AGRONOMIC MANAGEMENT AND BIOSTIMULANTS TO INCREASE CORN AND SOYBEAN PRODUCTIVITY

BY

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THESIS

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ABSTRACT

Corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] have been in production in the Corn Belt together since the 1920s. Improved cultivars and crop management has allowed for ever-increasing corn and soybean grain yields. Management factors that have played a big role in the yield increases are nitrogen (N) fertility, plant population, foliar protection, hybrid/variety, and additional nutrient fertility. Recently another management factor, biostimulants, is being evaluated as an option to further increase grain yield. Biostimulant products vary in their function, but all interact with at least one other management factor. The dependence of a biostimulant product's efficacy on these interactive effects can make it hard to predict the grain yield response of the crop. In an attempt to further understand the newest and fastest growing sector of the crop production agriculture industry, the objective of this research was to evaluate two different biostimulant products for their ability to increase growth and yield of soybean, and N use and productivity of corn. This research involved the following two studies.

Exploring the Interaction of Agronomic Management and a Plant Growth Regulator to Increase Productivity of Soybean

Biostimulants are becoming the newest products of interest in the agriculture industry, due to the wide array of influences on row crops and multiple modes of action. Consequently, there is a large array of products on the market with minimal government regulation because it is hard to discern how the biostimulant's effect would change with different interactions in the environment and when combined with management factors that impact soybean productivity. Therefore, understanding the interaction of a wide range of plant populations with a foliar-applied auxin inhibiting plant growth regulator (AIPGR) applied with or without foliar protection would aid farmers in increasing soybean grain yield productivity. The objective of this research was to quantify differential growth and yield responses of soybean grown at varying plant populations to an AIPGR biostimulant applied during vegetative or reproductive growth as well as with and without foliar protection. Soybean was sown at three locations in Illinois (northern, central, and southern) to achieve low (80,000 plants $acre^{-1}$), standard (140,000 plants $acre^{-1}$), and high (200,000 plants $acre^{-1}$) plant populations. In 2019, at each plant population, plants either received the AIPGR (GRAP GRAD; Agrocete, Cara-Cara, Paraná, BR) at the V5, R3, or V5 + R3 growth stage(s). Additionally, foliar protection (fungicide and insecticide) was applied to half of the plots that received the AIPGR at either the R3 growth stage or both V5 + R3 growth stages. The control plots at each plant population in 2019 included plants that received no foliar applications and plants that solely received foliar protection (fungicide and insecticide) application. In 2020, the same plant populations were used with foliar treatments implemented of an untreated control, AIPGR applications at the V5, R3, or V5 + R3 growth stage(s), with foliar protection on half of the AIPGR-treated plots. In both years, on average, the plant population producing the highest grain yield was 200,000 plants $acre^{-1}$, while the highest grain-yielding treatment was AIPGR applied at V5 + R3 with a foliar protection application. Increases in grain yield were typically driven by greater seed weight, in response to either greater plant population and/or foliar applications. Additionally, increasing the plant population tended to decrease the number of branches per plant. Overall, the results showed that the greatest yield was generated by planting 200,000 plants $acre^{-1}$, applying AIPGR at both the V5 + R3 growth stages, and applying foliar protection.

***Enhancing Nitrogen Uptake and Corn Productivity with* Azospirillum brasilense**

Of the many new biostimulant products, nitrogen-fixing bacteria (NFB) are possibly the most popular, because of the interest in reducing the need for the most-abundantly applied nutrient to a corn crop, nitrogen (N). Many NFB exist, but few studies have examined their effect on growth, nutrient uptake, and yield of corn. The objective of this research was to investigate the effects of a NFB, *Azospirillum brasilense*, in combination with varying N supplies at multiple environments on growth, N accumulation, and yield of corn. In 2019 and 2020, corn was grown in a five-rate N titration of 0, 50, 100, 150, and 200 lbs N acre^{-1} both with and without *A. brasilense* (GRAP NODa; Agrocete). Nitrogen treatments were broadcast and incorporated preplant as urea with *A. brasilense* applied in-furrow at the time of planting. In both years, the studies were conducted at three sites including northern, central, and southern locations, which have varying weather and native soil properties. Despite the varying environments, there were similar responses between locations and years. At the northern location in 2020, central location in 2019, and southern location in 2019 *A. brasilense* application tended to increase grain yield from 2 to 12 bushel acre^{-1} at the low N rates (0 and 50 lbs N acre^{-1}). However, there were also grain yield decreases in response to *A. brasilense* application, ranging from 3 to 22 bushel acre^{-1} in combination with the moderate N rates (100 and 150 lbs N acre^{-1}). The grain yield increases due to the application of *A. brasilense,* tended to be a result of greater kernel number at the lower N rates. Increases in plant N uptake at V8 and R6 in response to *A. brasilense* tended to be associated with the incidences of grain yield increase. Overall, the response of corn to the in-furrow applications of *A. brasilense* was highly dependent on the environment, with more positive responses at the higher-yielding environments.

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CHAPTER 1. EXPLORING THE INTERACTION OF AGRONOMIC MANAGEMENT AND A PLANT GROWTH REGULATOR TO INCREASE PRODUCTIVITY OF SOYBEAN

INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] in the United States was initially grown as a forage crop (Morse et al., 1950), but that purpose changed in 1917 when soybean seed began being processed for its oil and protein constituents for use as livestock feed (United Nations, 2016). The importance of soybean in the United States is seen by the increase of 88,623,000 acres planted to soybean from 1925 to 2017 (USDA-NASS, 2019), and during the 1970's, the United States began supplying two-thirds of the world's soybean demand (United Nations, 2016). Despite the large increase in acreage devoted to soybean, farmers tend to treat soybean primarily as a rotational crop, because, among other reasons, corn (*Zea mays* L.) grain yield is higher when the previous crop was soybean as compared to previously grown corn (Gentry et al., 2013). The continuous corn yield penalty incentivizes farmers to grow soybean as a rotational crop to corn, because the yield penalty associated with continuous corn makes growing soybean more profitable to growers. The soybean rotational incentive, however, is not often great enough for the farmer to manage soybean for higher yields, especially because of soybean's capability to obtain N from the atmosphere via biological N_2 fixation from their association with *Bradyrhizobium japonicum* (Harper, 1974).

An increasing number of progressive farmers are starting to intensively manage soybean and not view it simply as a rotational crop. How a crop is managed can be considered a spectrum, with some growers managing their crop more than others, but for the sake of simplicity, one can typically categorize soybean farmers as either standard or progressive. For the standard growers the main inputs are seed and herbicide, and the number of seeds to purchase is highly uncertain, because the ideal plant population varies based a number of environmental and management factors. There are numerous studies on soybean plant population, but no definitive answer as to the optimal plant population for greatest yields (Weber et al., 1966; Boquet, 1990; Edwards et al., 2005; Duncan, 1986). Varying results on the optimal population can be attributed to the different environments the trials were conducted in, especially the weather. High plant populations can lead to stagnant, warm, and humid air with less direct sunlight, which is an ideal environment for insects and disease, and which can decrease yield. Additionally, high plant populations can also cause yield loss from stem lodging (Boquet, 1990). Further issues when considering plant population include the risk of not enough plants to maximize the interception of sunlight, thereby potentially reducing grain yield (Weber et al., 1966).

Row spacing is often evaluated in conjunction with plant population, as these two agronomic practices are the easiest ways to increase the amount of light interception per area by the crop, and therefore increase potential yield. Increasing the amount of light interception can be accomplished by moving the rows closer together, but that change also results in earlier canopy closure creating a darker, warmer, and more humid environment with stagnant air, which is the ideal environment for diseases like white mold (Peltier et al., 2012). Despite the greater risk of yield reduction due to a more ideal environment for diseases, there are many reports of higher grain yields when soybean is grown in narrow rows (Bullock et al., 1998). The greater grain yield in narrow rows has been attributed to faster vegetative coverage and greater light interception before the seed–fill period (Bullock et al., 1998).

Farmers who intensively managing their soybean crops may apply fungicide and insecticide as either a reactive response to visible pest pressure or a proactive response in anticipation of pest pressure. The standard grower will typically have the reactive response while farmers who intensively manage soybean tend to have a proactive mentality. There is also the added benefit of the stay-green effect when applying strobilurin mode-of-action fungicides (Balba, 2007). This stay-green effect is when plants treated with the fungicide stay green longer than plants not treated, potentially allowing for longer leaf area duration, greater seed weight, and in turn higher grain yield. Because of the stay-green effect, grain yield increases have been observed from applying strobilurin mode-of-action fungicides even when no pest pressure was observed (Henry et al., 2011), although, the stay-green effect in the absence of lack of pest pressure does not always result in a grain yield increase (Viggers, 2019). Therefore, similar to plant population, the profitability of applying foliar protectants depends on the environment.

Another agronomic management practice employed by progressive soybean growers is fertilizing to ensure that the plants have sufficient levels of macronutrients. Phosphorus (P) is one of the three primary essential macronutrients that is needed in large quantities, notably 43 lbs P_2O_5 per acre for a soybean crop yielding 60 bushel per acre (Bender et al., 2015). That amount is crucial for grain yield, as approximately 80% of the total phosphorus taken up by the plant is removed from the field in the grain at harvest. The high demand for P can be hard to fulfill, as phosphorus is immobile in the soil, making it necessary for the plant's root to intercept it for plant uptake (Maathuis, 2009). However, the majority of growers do not apply phosphorus to soybean crops as they rely on the residual P remaining from the previous year's fertilization to the corn crop.

While applying foliar protectants and fertilizing soybean with P is becoming more common, the least utilized of the agronomic management factors is biostimulants, in part because they are relatively new. Biostimulants were recently defined in the 2018 Farm Bill as "a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield" (Agriculture Improvement Act, 2018). One of the categories of biostimulants is plant growth regulators (PGR). The use of PGRs is not new to agriculture as they have been widely utilized in specialty crops such as grapes (Rane et al., 2017), and synthetic auxins have been used for decades for weed control in row crops (Grossmann, 2010). However, the use of PGRs for greater grain yield is relativity new for soybean production.

The five main classes of plant hormones produced and used by plants include auxin, cytokinin, ethylene, gibberellin, and abscisic acid (Kende and Zeevaart, 1997). The use of PGRs to affect auxin activity in the plant is common due to the large role of auxin in the coordination of plant growth processes and its interaction with other PGR groups, especially cytokinin. The interaction of auxin and cytokinin levels is known to regulate organogenesis, root meristem development, early meristem formation, and shoot meristem development (Su et al., 2011). Auxin is also known to mediate the growth of lateral buds by influencing the production of cytokinin (Nordström et al., 2004). Furthermore, auxin is responsible for the phenomenon of phototropism, as it is transported asymmetrically up the plant, causing one side of the plant to grow longer and turn towards the light (Christie et al., 2011). Therefore, using an auxin-inhibiting PGR (AIPGR) to temporarily inhibit the action of auxin, may in turn, promote greater branching, lateral growth (i.e., shorter plants), and result in increased grain yield.

Foliar protectants and the selected auxin-inhibiting PGR need to be applied at certain plant growth stages for greatest effectiveness. An understanding of soybean growth stages can help to determine their optimal time of application. In the indeterminate soybean variety that was utilized for this study, the reproductive stages overlap with vegetative stages. The first vegetative growth stage is emergence (VE growth stage) when the cotyledons are above the soil surface followed by the VC growth stage when unifoliate leaf edges are unrolled and no longer touching (Purcell and Ashlock, 2014). Then following the VC growth stages, the growth stage is determined by the number of the highest node with a fully developed leaf, defined as when the leaf edges are unrolled and are no longer touching. The soybean plant is staged in the vegetative growth stages until the first reproductive development stages (R1 and R2) are identified by flowering on the main stem (Purcell and Ashlock, 2014). After that, the development stages continue through pod set (R3 and R4), seed set (R5 and R6), and maturation (R7 and R8) determined by when the pods have reached their mature color (Purcell and Ashlock, 2014).

As soybean growth is not rapid enough to suppress weed pressure, there is typically a post-emergence herbicide application when the trifoliate at the 5th node is fully developed (V5 growth stage). This time is normally the final herbicide application, as the plants will be able to close the crop canopy soon after that time, preventing younger weeds from incepting direct sunlight, and decreasing their competition pressure. Typically, the last management practice is to apply foliar protection between the second and fourth reproductive development stages. Foliar protection is typically applied at this time to protect the leaves that are present, and therefore extending leaf area duration, to increase the seed weight and ultimately increase grain yield.

The objective of this research was to determine the interactive effects of application timings of an auxin-inhibiting plant growth regulator (AIPGR) biostimulant, plant population, and foliar protection on the growth and grain yield of soybean. To accomplish this objective, the AIPGR biostimulant called GRAP GRAD (Agrocete, Cara-Cara, Paraná, Brazil), was applied at two plant growth stages (V5 and/or R3) to plants grown at three plant populations (80,000, 140,000, or 200,000 plants/acre) either with or without insecticidal plus fungicidal foliar protection.

MATERIALS AND METHODS

Field Characteristics

The trial was implemented in the 2019 and 2020 growing seasons at three locations across the state of Illinois. In 2019, this study was conducted at the Crop Science Research and Education Center (CSREC) located at the University of Illinois Urbana-Champaign and at two off-site locations: at Yorkville, IL, in the northern part of the state and Ewing, IL, in the southern part of the state. Soil types differed between the locations with the Yorkville location consisting of a Drummer silty clay loam soil type, the Champaign location consisted of a Flanagan silt loam, and the Ewing location consisting of a Hoyleton silt loam. In 2020, the study was implemented at Yorkville and Champaign, as well as an alternate southern location at Nashville, IL. The soil types in 2020 were a Drummer silty clay loam at Yorkville, Flanagan silt loam at Champaign, and a Darmstadt-Coulterville silt loam at Nashville. Pre-plant soil samples (0-6 inches deep) were obtained from plot areas prior to planting and analyzed (A & L Great Lakes Laboratories, Fort Wayne, IN) to confirm soil fertility levels (Table 1.1).

Herbicide Applications

In 2019, the locations were maintained in a weed-free environment primarily by using a pre-emergence application of pyroxasulfone (3-[[[5-[difluoromethoxy]-1-methyl-3-[trifluoromethyl]-1H-pyrazol-4-yl]methyl]sulfonyl]-4,5-dihydro-5,5-dimethylisoxazole) known as Zidua (BASF Corporation, Ludwigshafen, Germany) at a rate of 3 oz acre^{-1} and flumioxazin (2-[7-fluoro-3,4-dihydro-3-oxo-4-[2-propynyl]-2H-1,4-benzoxazin-6-yl]-4,5,6,7-tetrahydro-1H-isoindole-1,3[2H]-dione) + pyroxasulfone (3-[[[5-(difluoromethoxy)-1-methyl-3-(trifluoromethyl)-1H-pyrazol-4-yl]methyl]sulfonyl]-4, 5-dihydro-5,5-dimethylisoxazole) known as Fierce (Valent, Walnut Creek, CA) at a rate of 0.5 oz acre^{-1} at Yorkville, IL; and a pre-emergence application of S-metolachlor (2-chloro-N-[2-ethyl-6-methylphenyl]-N-[2-methoxy-1-methylethyl] acetamide) + metribuzin (4-amino-6-tert-butyl-3-methylsulfanyl-1,2,4-triazin-5-one) known as Boundary 6.5G (Syngenta, Basel, Switzerland) at a rate of 20 oz acre^{-1} with sulfentrazone (*N*-[2,4-dichloro-5-[4-(difluoromethyl)-3-methyl-5-oxo-1,2,4-triazol-1-yl]phenyl]methanesulfonamide) + cloansulam-methyl (3-chloro-2-[(5-ethoxy-7-fluoro-[1,2,4]triazolo[1,5-c]pyrimidin-2-yl)sulfonylamino]benzoic acid) known as Authority 1st (FMC, Philadelphia, PA) at a rate of 5 oz acre^{-1} at Ewing, IL. Post-emergence applications at the V3 growth stage were also used to maintain a weed free environment using pyroxasulfone + fluthiacet-methyl (methyl-2-[2-chloro-4-fluoro-5-[(3-oxo-5,6,7,8-tetrahydro-[1,3,4]thiadiazolo[3,4-a]pyridazin-1-ylidene)amino]phenyl]sulfanylacetate**)** known as Anthem Maxx (FMC, Philadelphia, PA) at a rate of 3.5 oz acre^{-1}, and glyphosate (N-phosphonomethyl glycine, in the form of a potassium salt), known as RoundUp WeatherMaxx (Bayer, St. Louis, MO) at a rate of 32 oz acre^{-1} at Yorkville and Champaign, IL.

In 2020, the locations were also maintained in a weed-free environment. There were pre-emergence applications of Boundary 6.5G at a rate of 20 oz acre^{-1}, Authority 1st at a rate of 5 oz acre^{-1}, and glufosinate (2-amino-4-[hydroxy(methyl)phosphoryl]butanoic acid) known as Liberty (Bayer, St. Louis, MO) at a rate of 40 oz acre^{-1} at Yorkville and Nashville. At Champaign, the pre-emergence herbicide application consisted of pyroxasulfone + imazethapyr ((±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5- oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid) + saflufenacil (N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3, 6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide) known as Zidua Pro (BASF Corporation, Ludwigshafen, Germany) at a rate of 6 oz acre^{-1} and Boundary 6.5G at a rate of 12 oz acre^{-1}. Post-emergence applications were also used for weed-free environments at all locations.

At Yorkville and Nashville, at the V1 growth stage, there was a post-emergence herbicide application of diglycolamine salt of dicamba (3,6-dichloro-o-anisic acid) known as Xtendimax (Bayer, St. Louis, MO) at a rate of 22 oz acre^{-1}, S-metochlor + atrazine (1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) known as Dual II Magnum (Syngenta, Basel, Switzerland) at a rate of 16 oz acre^{-1}, glyphosate known as RoundUp WeatherMaxx (Bayer, St. Louis, MO) at a rate of 32 oz acre^{-1}, Intention Advanced (FS Growmark, Bloomington, IL) drift control at a rate of 5% v v^{-1}, and Class Act (WinField United, St. Paul, MN) surfactant at a rate of 19.2 oz acre^{-1}. At Champaign the post-emergence application at the V1 growth stage consisted of acetochlor (2-chloro-N-(ethoxymethyl)-N-(2-ethyl-6-methylphenyl)acetamide 2-chloro-2'-methyl-6'-ethyl-N-ethoxymethyl-acetanilide) known as Warrant (Bayer, St. Louis, MO) at a rate of 56 oz acre^{-1}, RoundUp WeatherMaxx at a rate of 32 oz acre^{-1}, and fluazifop-p-butyl (butyl (R)-2-[4-[[5-(trifluoromethyl)-2- pyridinyl]oxy]phenoxy]propanoate) known as Fusilade (Syngenta, Basel, Switzerland) at a rate of 4 oz acre^{-1}. To keep Nashville a weed-free environment, a second post-emergence application at the V4 growth stage of Anthem Maxx at a rate of 3.5 oz acre^{-1}, and RoundUp WeatherMaxx at a rate of 32 oz acre^{-1} was necessary.

Agronomic Management

Corn was the previous crop and conventional tillage was used in both seasons. A soybean variety responsive to management was planted to achieve populations of 80,000, 140,000, and 200,000 plants acre^{-1}. The variety planted differed between the two growing seasons, with Asgrow 37X9 (Bayer, St. Louis, MO) utilized in 2019 and Asgrow 34X6 (Bayer, St. Louis, MO) used in 2020. The variety in 2019 was in the 3.7 maturity group while the variety in 2020 was in the 3.4 maturity group. Plots were planted on 30-inch row spacing with a Seed Pro 360 planter (ALMACO, Nevada, IA) on 10 June 2019 at Yorkville, 3 June 2019 at Champaign, and 11 June

2019 at Ewing. In the following year, plots were planted on 20-inch row spacing on 4 June 2020 at Yorkville, 13 May 2020 at Champaign, and 7 June 2020 at Nashville. Further agronomic management of the trial in both years included the entire trial receiving phosphorus and sulfur as 188 lbs acre^{-1} MicroEssentials S10 (12-40-0-10S, The Mosaic Company, Tampa, FL) broadcast preplant with a 10T-Series drop spreader (Gandy, Owatonna, MN).

Treatment Applications

Applications were designed to evaluate an auxin-inhibiting plant growth regulator (AIPGR), known as GRAP GRAD (Agrocete, Cara-Cara, Paraná, Brazil), for its role in inhibiting auxin production for a short period of time (3-5) days and productivity of soybean (Luiz Michelini, personal communication, 2018) (Tarik Yoshida, personal communication, 2020). In both years, this product was applied at 10.3 oz acre^{-1} along with Super Gun (Agrocete, Cara-Cara, Paraná, Brazil), a surfactant, at 0.064 oz gallon^{-1} at either the V5 growth stage, the R3 growth stage, or both and compared to untreated control plots (Table 1.2). Based on results from 2019, a treatment alteration was made in 2020 where in 2019 a treatment solely received foliar protection, but in 2020 received AIPGR at the V5 growth stage and foliar protection. Foliar protection was also applied to a subset of the plants at the R3 growth stage and included alpha-cypermethrin (mixture of (S)-α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and (R)-α-cyano-3-phenoxybenzyl (1S,3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) known as Fastac CS (BASF Corporation, Ludwigshafen, Germany) at a rate of 3.8 oz acre^{-1} plus fluxapyroxad (1H-Pyrazole-4-carboxamide, 3-(difluoromethyl)- 1-methyl-N-(3',4',5'-trifluoro[1,1'-biphenyl]-2-yl)) + pyraclostrobin (carbamic acid, [2-[[[1-(4-chlorophenyl)- 1H-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-, methyl ester) known as Priaxor Xemium (BASF Corporation, Ludwigshafen, Germany) at a rate of 8 oz acre^{-1}. All treatment applications were applied to the center two rows of each designated plot using a pressured CO_2 backpack sprayer with water as the carrier for the total spray volume of 15 gallon acre^{-1} application rate. The boom was equipped with XR8002 nozzles (TeeJet, Glendale Heights, IL) to provide 80º spray pattern in a flat fan pattern to provide even and full coverage across the center two plot rows. In 2019, the AIPGR treatments for the V5 growth stage applications occurred on 22 July 2019 at Yorkville, 9 July 2019 at Champaign, and 17 July 2019 at Nashville. The R3 growth stage applications occurred on 9 August 2019 at Yorkville, 31 July 2019 at Champaign, and 7 August 2019 at Ewing. In the following year, the AIPGR treatments for the V5 growth stage applications occurred on 7 July 2020 at Yorkville, 25 June 2020 at Champaign, and 10 July 2020 at Nashville. The R3 growth stage applications occurred on 19 August 2020 at Yorkville, 20 July 2020 at Champaign, and 18 August 2020 at Nashville.

Measured Parameters

In 2019, ten days after the R3 application in Champaign (8 August), the average numbers of pods, nodes, and branches were determined by removing 10 representative plants from the center two rows of each plot and then manually counting each parameter.

Stem diameter and plant height were measured at the R5 plant growth stage to assess any AIPGR, planting population, or foliar protection effects on plant growth at all locations in 2019. The measurements were obtained from a random section of 10 plants in one of the two center rows of each plot. A calibrated caliper (Westward, Saltash, United Kingdom) was used to measure stem diameter just below the first node, and a meter stick was used to measure plant height from the base of the plant at the soil surface to the newest node. These measurements were taken on 20 August, 10 August, and 16 August at Yorkville, Champaign, and Nashville, respectively.

At the R6 growth stage at the Champaign location in 2020, five consecutive representative plants were removed from one of the center two rows of every plot on 2 September, and the number of branches and raceme branches were recorded for each plant. Raceme branches are small branches with a trifoliate that arise from a position on the flower raceme rather than from a stem axillary bud.

Upon completion of the growth cycle, the middle two rows of each plot were harvested with a combine (SPC40, ALMACO, Nevada, IA) to determine grain yield, with values adjusted to 13% moisture. The harvest dates in 2019 were 9 November, 25 October, and 14 October, at Yorkville, Champaign, and Ewing, respectively. In the second year of this study, the harvest dates were 7 November 2020 at Yorkville, 6 October 2020 at Champaign, and 8 October 2020 at Nashville. The combine also collected subsamples of the harvested grain that were evaluated for grain quality (protein and oil concentrations at 13% grain moisture) by utilizing near-infrared transmittance spectroscopy (NIT) with a grain analyzer (Infratec 1241, Foss, Eden Prairie, MN). The grain quality data is presented in supplemental tables A1.1 to A1.6, but will not be discussed in detail, but for the most part grain protein concentration tended to increase with higher plant population and grain oil concentration tended to decrease. Average seed weights were estimated based on a representative subsample of 300 seeds and adjusted to 0% moisture. Seed number on a per-acre basis was obtained from dividing total grain weight by the average seed weight.

Experimental Design and Statistical Analysis

In 2019 and 2020, treatments were arranged in a split-plot randomized complete block design with six replications and 21 treatments for a total of 126 plots at each location (grand total of 756 plots). Each plot was four rows wide and 36 ft in length with 30-inch row spacing in 2019 and 20-inch row spacing in 2020. Statistical analysis was conducted using PROC MIXED in SAS (version 9.4; SAS Institute, Cary, NC). The AIPGR treatment and plant population rate were considered fixed effects, with location and treatment by plant population as a random factor in the model. Significance was declared at $P \leq 0.10$. PROC GLM of SAS was utilized to conduct the Brown-Forsythe test of the Levene test for homogeneity of variance on the errors and significance was declared at $P \leq 0.05$. PROC UNIVARIATE of SAS was used to determine possible outliers and assess the normality of the errors, with significance declared at $P \leq 0.01$. In addition to the Shaprio-Wilk test, QQ plots and histograms were studied to determine normality of the errors, when the Shapiro-Wilk tests were significant. With homogeneity of variance and normality assumptions met, the locations were analyzed separately by year due to differing treatments, row spacing, varieties, and measurements.

RESULTS AND DISCUSSION

Effects of AIPGR in Multiple Plant Populations With and Without Foliar Protection in 2019

Soil Characteristics

Preplant composite soil test values varied across the locations (Table 1.1). There was greater inherent soil fertility the further north the plots were in Illinois. Native soil organic matter and CEC levels also had an increasing trend from the southern (Ewing or Nashville) to the northern (Yorkville) locations. All elements are considered as adequate for optimal productivity except for K at the Ewing site (Table 1.1).

Weather

Due to excessive spring precipitation (Table 1.3), these trials were planted a month later than normal, leading to a shorter than optimal growing season. The Yorkville location experienced the most rainfall, receiving 16.8 inches more precipitation throughout the season than its 30-year average (Table 1.3). Overall, the Champaign and Ewing locations had excess precipitation early in the year but were warmer and drier than average July through September. Champaign was drier than average in August during the reproductive development stage initiation, while Ewing was much drier than average in September during grain fill (Table 1.3).

Height and Diameter

At all three locations, plant height at the R5 growth stage tended to increase with the plant population, while stem diameter markedly decreased (Tables 1.4 and 1.5). The difference in plant height was apparent as early as the R1 growth stage (Figure 1.1). Taller plants have been found to occur with higher plant populations, due to the plants sensing greater competition horizontally, causing the plants to grow more vertically (Weber et al., 1966; Hoggard et al.,1978). Although foliar protection increased stem diameter at the lowest population at Yorkville (Table 1.5), the effect of AIPGR applications and foliar protection at the other locations and populations was variable.

Pod, Node, and Branch Counts

Branch numbers in the current study confirmed previous reports that plants grown under lower plant populations produce more branches per acre (Lueschen and Hicks, 1977; Weber et al., 1966) (Table 1.6). In a similar manner, pod counts in 2019 showed that when fewer plants were grown, those plants produced more pods per plant due to the branches producing more pods at the low plant population (80,000 plants acre^{-1}), as the number of pods on the main stem was relativity similar across plant populations compared to the total number of pods per plant (Table 1.6). The treatment generating the greatest number of total pods per plant was AIPGR application at V5 + R3 at the lowest planting population (80,000 plants acre^{-1}). Applications containing AIPGR at R3 (both with and without foliar protection) also increased pods per branch at the lowest planting population (Table 1.6). Neither application of AIPGR nor foliar protection affected branching, total pods per plant, pods per branch, or pods per main stem at either the intermediate or high planting populations, although all parameters decreased as the planting population increased (Table 1.6).

Grain Yield and Yield Components

Plants at the Yorkville location produced the highest yields of all three locations (Tables 1.7, 1.8, and 1.9). Greater yields were especially evident at the two higher planting populations at Yorkville in response to AIPGR being applied at both V5 and R3 in combination with foliar protection, and AIPGR being applied at R3 with foliar protection (Table 1.7). The grain yield increases observed from the foliar treatments can be attributed to greater grain fill, noted by the greater seed weight, as a result of healthier plants and longer light interception. Earlier studies reported an increase in grain yield from the application of foliar protection without noticable pest pressure, suggesting that the grain yield increase was a result of increased plant health (Kandel et al., 2016), and an increase in grain yield from higher plant density (Edwards et al., 2005). However, grain yields of Yorkville were not significantly increased by the foliar treatment and in some cases were significantly lower than the untreated control (Table 1.7). At Champaign, grain yields were increased by the V5 + R3 AIPGR applications at the intermediate population (140,000 plants acre^{-1}), while at the highest population (200,000 plants acre^{-1}), the R3 application time (with or without the V5 AIPGR application) generated the greatest yield increases, especially in combination with foliar protection (Table 1.8). Also at Champaign, as well as at Ewing, there was a tendency to increase yields by increasing the planting population (Tables 1.8 and 1.9). Grain yield responses to AIPGR application at Ewing were similar to Yorkville, where the three highest-yielding treatments resulted from applying AIPGR at R3 with foliar protection, and AIPGR at V5

+ R3 with or without foliar protection, and these increases were primarily observed at the lowest and highest planting populations. At all locations, the addition of foliar protection tended to increase grain yield by generating healthier plants and fostering greater seed weight (Henry et al., 2011). Across all locations, there was a tendency for plant population increases of 60,000 plants acre^{-1} to increase the grain yield by roughly 2 bushel acre^{-1} (Tables 1.7 - 1.9). Other research has shown that increased plant population can either increase or decrease grain yield, depending on the environment (Lueschen and Hicks, 1977). Similarly, Weber et al. (1966) reported that a change in the environment (row spacing) affected grain yield and interacted with plant population to affect grain yield. The grain yield increase in response to plant population in 2019 was the result of greater grain fill and heavier average seed weights, as the seed number per area was similar, regardless of the plant population (Tables 1.7-1.9).

2019 Conclusions

In general, applications of AIPGR at R3 gave the most consistent yield increases, and adding the V5 AIPGR application and foliar protection boosted yields further. The management factors that achieved the greatest grain yield was 200,000 plants acre^{-1} with the dual application of the AIPGR with foliar protection. Since, the highest plant population (200,000 plant acre^{-1}) produced taller plants with a smaller stem diameter, the plants may have been more susceptible to abiotic stress and grain yield loss, and therefore, the plants at the highest population benefitted the most from both foliar applications of the AIPGR and foliar protection.

Effects of AIPGR in Multiple Plant Populations With and Without Foliar Protection in 2020

Trial Redesign

The trial was redesigned in 2020 to better evaluate the interaction of AIPGR and foliar protection by adding a treatment of a V5 application of the AIPGR. With all applications of AIPGR being with and without foliar protection, there was no longer the need to quantify the value of foliar protection as a separate application. Having noticed raceme branches in 2019 but not quantifying, raceme branches were counted at the R6 development stage in 2020. The row spacing was also narrowed from 30 to 20-inches because farmers intensively managing their crops would be the most likely to utilize the AIPGR and because 20-inch row spacing tends to increase grain yields compared to 30-inch row spacing (Bullock et al., 1998). Furthermore, a soybean cultivar of a relative maturity group more suitable (3.4 maturity group) for all three locations was utilized in 2020 compared to the 3.7 maturity group soybean that was used in 2019.

Soil Characteristics

Preplant composite soil samples were taken to analyze for organic matter, cation exchange capacity, pH, phosphorus, potassium, calcium, magnesium, sulfur, zinc, manganese, and boron levels (Table 1.1). Similar to 2019, soil organic matter, cation exchange capacity, phosphorus, and potassium tended to be higher the further north in Illinois. Conversely, the soil pH tended to be higher the further south in Illinois in the preplant soil samples (Table 1.1). For the 2020 research sites, soil test levels of P and K tended to be lower than optimal at both Champaign and Nashville.

Weather

Excessive early-season precipitation at Yorkville and Champaign and untimely rains at Nashville (Table 1.3) resulted in soil conditions that were not fit for planting and causing these trials to be planted a month later than normal. Although seasonal precipitation and average temperatures were close to the 30-year averages at Yorkville and Champaign, both sites experienced unique weather challenges that negatively affected yield. At Champaign, a hail storm on July 11 caused up to 50% leaf defoliation, and at Yorkville, a derecho wind storm caused minor stem lodging. The Yorkville and the Champaign sites also experienced a lack of precipitation during the month of August that negatively affected seed-filling and Nashville was abnormally dry during September (Table 1.3).

Branch and Raceme Branch Counts

The number of plant branches and raceme branch development was only conducted at Champaign (Table 1.10). As expected, branch number successively decreased with increases in plant population, similar to previous reports (Lueschen and Hicks, 1997) (Table 1.10). When AIPGR was applied at V5, and to a lesser extent at R3, there was a tendency for AIPGR alone to decrease branching at each plant population. The data contrasted with the theory of AIPGR's mode of action which was to increase grain yield by enhancing branching (Luiz Michelini, personal communication, 2018) (Tarik Yoshida, personal communication, 2020). Further conflicting knowledge is that branching typically starts around the V5 growth stage and as an indeterminate soybean variety was utilized branching could continue through the reproductive stages (Egli and Leggett, 1973). This decrease of branching from AIGPR application, however, was reversed when AIPGR was applied with foliar protection. In contrast to branching, foliar treatment with AIPGR application tended to increase raceme branches, while adding foliar protection alone deceased raceme branching, and these effects were especially apparent at the lowest plant population (Table 1.10). The temporary inhibition of auxin production allowed the lateral growth nodes to be promoted enough where raceme branches were able to be established, but that was negated by foliar protection. Although the mechanism by which auxin specifically inhibits auxiliary bud growth is unknown (Leyser, 2003), we believe that the AIPGR allowed for the promotion of branching long enough for a raceme branch to initiate. Although there was a tendency for the sole application of AIPGR to increase raceme branches the difference was not statistically significant (Table 1.10).

Grain Yield and Yield Components

The grain yield response to plant population, foliar protection, and AIPGR application differed depending on the location (Tables 1.11-1.13). The main effect of plant population (averaged over the foliar treatments) significantly affected yields at Yorkville and Champaign, but not at Nashville (Tables 1.11-1.13). At Yorkville, grain yield increased by about 3 bushels acre^{-1} with each 60,000 plant acre^{-1} increase, and the highest yield was achieved with 200,000 plants acre^{-1} (Table 1.11). Conversely at Champaign, yield increased by almost 10 bushels acre^{-1} as the plant population increased from 80,000 to 140,000 plants acre^{-1}, but by only 2.5 bushel acre^{-1} with the increase from 140,000 to 200,000 plants acre^{-1} (Table 1.12). These yield responses to population are reflected in the seed number, and the plant population was a large significant source of variation (<0.0001) for seed number at Champaign (Table 1.12). While seed number increased with greater plant population at Champaign, seed weights remained relatively constant, regardless of plant population. At Nashville, which was the lowest yielding site, neither plant population nor the foliar treatments significantly affected yield (Table 1.13). Nashville plots did not exhibit an increase in grain yield with the increase in plant population due to the lack of the yield-based response in increased seed number that was observed at Yorkville and Champaign. The dry weather at the end of the growing season at Nashville likely inhibited the full potential of the plants to maximize seed weights, similar to previous reports by Frederick et al. (2001) (Table 1.3 and 1.13). However, plant population was still a significant source of variation for seed weight at Nashville. At all locations, each increase in plant population generated greater seed weight, with the solitary exception of the plant population increase of 140,000 plants acre^{-1} to 200,000 plants acre^{-1} at Champaign (Tables 1.11-1.13).

Averaged over plant populations, the foliar treatments (either protection or AIPGR) significantly affected yield only at Champaign, where all foliar treatments led to numerically greater grain yields than the untreated control (Table 1.12). These yield increases at Champaign were especially apparent when the foliar treatments included foliar protection with the AIPGR, resulting in 4 to 5 more bushels per acre compared to the untreated control. Although not statistically significant, AIPGR application at V5 + R3 along with foliar protection and at 140,000 plants acre^{-1} increased yield by 5.4 bushel acre^{-1} over the untreated control at Yorkville. These yield increases can be attributed to foliar protection creating healthier plants and AIPGR promoting greater photosynthesis, both aiding in greater grain fill; thereby boosting seed weight on average by 7 mg seed^{-1} more than the untreated control (Table 1.11) This data in agreement with Henry et al. (2011) who reported grain yield increases from the application of foliar protection despite the lack of need to protect the foliar vegetation due to minimal pest pressure.

The further north the location the greater the yield response to the highest plant population (200,000 plants acre^{-1}) (Tables 1.11-1.13). This yield response result is potentially due to the growing season being shorter the further north the location, and when the growing season is shortened because delayed planting, soybean is recommended to be sown at a higher seeding rate (Oplinger and Philbrook, 1992). Additional plants are needed later in the growing season to keep the plant population from being the limiting factor of grain yield. This tendency is seen in the data with the smallest yield response at Nashville and the greatest grain yield response to the same incremental plant population increase at Yorkville (Tables 1.11-1.13).

2020 Conclusion

The hail at Champaign and relatively dry conditions during seed-filling at Nashville played important roles in the yield responses to plant population by affecting seed number at Champaign and seed weight at Nashville. At Champaign, the increased raceme branches theorized to have been promoted by the AIPGR did not significantly increase seed weight. Conversely, across all locations, foliar protection tended to produce the greatest seed weight compared to the same treatment without foliar protection. At all locations, greater plant population also tended to increase seed weight, but also caused greater grain yield increases than the foliar treatments, especially at the northern locations (Yorkville and Champaign).

TABLES AND FIGURE

Table 1.1 Pre-plant soil properties and Mehlich 3-extraction-based mineral test results for AIPGR trials conducted at Yorkville, Champaign, Ewing, and Nashville, Illinois in 2019 and 2020. Organic matter is abbreviated as OM, and cation exchange capacity is abbreviated as CEC.

Year	Location	OM	CEC	pH	P	K	Ca	Mg	S	Zn	Mn	Fe	Cu	B
		%	meq/100g	unit	ppm									
2019	Yorkville	4.6	22.1	5.8	105	202	2224	676	11	3	34	195	3	0.5
	Champaign	3.2	15.3	6.4	44	142	2069	411	6	2	46	159	2	0.4
	Ewing	2.0	8.8	6.9	28	63	1524	110	5	1	199	157	1	0.2
2020	Yorkville	4.8	23.2	6.1	24	121	2737	675	10	2	24	113	3	0.9
	Champaign	3.7	16.1	6.9	14	90	2277	514	7	2	52	123	2	0.6
	Nashville	2.2	8.5	6.5	17	87	1229	107	8	1	135	178	1	0.1

Table 1.2 Twenty-one treatments in a single year used to evaluate the effect of AIPGR application time and agronomic management on soybean growth and productivity at Yorkville, Champaign, Ewing, and Nashville, Illinois in 2019 and 2020.

Population	AIPGR Application†	Foliar Protection‡	Year(s) Treatment Implemented
plants acre^{-1}			
80,000	-	-	2019, 2020
	-	Added	2019
	V5	-	2019, 2020
	V5	Added	2020
	R3	-	2019, 2020
	R3	Added	2019, 2020
	V5 + R3	-	2019, 2020
	V5 + R3	Added	2019, 2020
140,000	-	-	2019, 2020
	-	Added	2019
	V5	-	2019, 2020
	V5	Added	2020
	R3	-	2019, 2020
	R3	Added	2019, 2020
	V5 + R3	-	2019, 2020
	V5 + R3	Added	2019, 2020
200,000	-	-	2019, 2020
	-	Added	2019
	V5	-	2019, 2020
	V5	Added	2020
	R3	-	2019, 2020
	R3	Added	2019, 2020
	V5 + R3	-	2019, 2020
	V5 + R3	Added	2019, 2020

† All AIPGR applications consisted of Super Gun at a rate of 0.064 ounces per 1 gallon of water and AIPGR at a rate of 10.3 oz/acre at the designated growth stage.

‡ Priaxor Xemium fungicide at a rate of 8 oz/acre with Fastac CS insecticide at a rate of 3.8 oz/acre added at the R3 application only. -, No foliar protection applied.

Table 1.3 Precipitation and temperature during the production season at Yorkville, Champaign, Ewing, and Nashville, IL in 2019 and 2020 compared to the 30-year average. Values were obtained from the Illinois State Water Survey.

	Year							
	2019				2020			
	Precipitation		Temperature		Precipitation		Temperature	
Month	Season	30-Year Average	Season	30-Year Average	Season	30-Year Average	Season	30-Year Average
	—— inches ——		—— °F ——		—— inches ——		—— °F ——	
				Yorkville				
April	4.8	3.9	48	50	3.6	3.0	46	49
May	8.4	3.6	58	61	6.1	3.8	58	60
June	2.6	3.8	69	70	3.3	3.8	61	70
July	2.8	3.2	75	72	4.4	3.2	74	72
August†	4.4	3.4	69	70	0.9	3.4	58	70
September	12.0	2.7	67	63	5.1	3.0	61	63
October	5.1	2.7	48	52	2.2	2.8	47	51
Total/Average	40.1	23.3	62	63	25.6	23.0	58	62
				Champaign				
April	5.3	3.7	53	53	5.3	3.7	50	53
May	5.2	4.7	64	63	4.7	4.7	61	63
June	3.7	4.4	72	73	5.8	4.4	74	72
July‡	2.3	4.2	77	75	4.6	4.1	77	75
August	2.1	3.4	74	74	1.3	3.4	73	74
September	3.3	3.1	72	67	2.9	3.1	65	67
October	5.0	3.2	54	55	2.4	3.3	52	55
Total/Average	26.9	26.7	67	66	27.0	26.7	65	66
		Ewing				**Nashville**		
April	7.1	4.8	58	58	4.7	4.4	54	56
May	7.0	4.7	67	67	4.3	4.9	64	66
June	3.5	4.0	73	75	4.0	3.9	77	74
July	2.1	3.6	79	78	9.1	3.3	80	77
August	2.2	3.1	76	76	7.5	3.3	75	75
September	0.3	3.5	75	69	0.6	2.9	68	67
October	8.0	3.5	57	58	5.1	2.9	55	57
Total/Average	30.2	27.2	69	69	35.3	25.6	68	67

† August 10th, 2020 recorded a derecho with winds ranging 70 -126 mph.

‡ July 11th, 2020 recorded 0.75 – 1.5 inch sized hail.

Figure 1.1 Visual difference in growth of plants sown at 200,000 plants acre^{-1} (left) and 80,000 plants acre^{-1} (right). Picture of R1 plants are at Champaign, Illinois, in 2019.

Table 1.4 Effect of foliar treatments and plant population on plant height at the R5 growth stage of soybean grown at all three locations in IL in 2019.

Foliar Treatments		**Plant Population,** plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		centimeters plant^{-1}			
			Yorkville		
None	-	92	101	95	96
None	Added	87	93	94	92
V5	-	92	92	90	91
R3	-	93	91	95	93
R3	Added	89	94	90	92
V5 + R3	-	87	93	91	90
V5 + R3	Added	90	97	99	95
Average		90	94	94	
			Champaign		
None	-	85	91	87	87
None	Added	87	91	90	90
V5	-	88	90	88	90
R3	-	91	88	90	88
R3	Added	88	92	92	91
V5 + R3	-	86	91	87	88
V5 + R3	Added	83	90	92	88
Average		87	91	90	
			Ewing		
None	-	78	80	85	81
None	Added	81	82	87	84
V5	-	82	84	89	85
R3	-	80	81	86	83
R3	Added	81	81	85	82
V5 + R3	-	80	80	90	83
V5 + R3	Added	82	84	85	84
Average		81	82*	87*	

Source of Variation	Yorkville	Champaign	Ewing
		p-value	
Foliar Treatments	0.0419	0.2860	0.1306
Plant Population	0.2981	0.6842	0.0120
Plant Pop. *x* Foliar Treat.	0.4539	0.2067	0.5205

*Denotes a significant main effect compared to no treatment or 80,000 plants acre^{-1}.

Table 1.5 Effect of foliar treatments and plant population on stem diameter at the R5 growth stage of soybean grown at all three locations in IL in 2019.

Foliar Treatments		Plant Population, plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		millimeters plant^{-1}			
			Yorkville		
None	-	9.6	7.8	5.7	7.7
None	Added	9.8	7.3	5.9	7.7
V5	-	9.5	7.7	6.3	7.8
R3	-	9.4	6.8	6.6	7.6
R3	Added	9.8	7.0	6.3	7.7
V5 + R3	-	9.0	7.6	6.7	7.8
V5 + R3	Added	9.2	7.6	6.6	7.8
Average		9.5	7.4	6.3	
			Champaign		
None	-	8.7	6.9	5.7	6.9
None	Added	7.5	6.8	4.9	6.6
V5	-	7.7	6.3	5.4	6.4
R3	-	8.1	6.4	5.4	6.9
R3	Added	7.7	6.4	5.4	6.6
V5 + R3	-	7.9	6.5	4.9	6.4
V5 + R3	Added	8.2	6.5	5.1	6.6
Average		8.4	6.2*	5.4*	
			Ewing		
None	-	8.3	7.3	6.2	7.3
None	Added	8.9	7.0	6.1	7.3
V5	-	8.6	6.9	6.0	7.2
R3	-	8.5	6.1	5.9	6.8
R3	Added	8.9	7.1	5.4	7.1
V5 + R3	-	8.7	6.8	6.1	7.2
V5 + R3	Added	8.4	6.9	5.6	7.0
Average		8.6	6.9*	5.9*	

Source of Variation	Yorkville	Champaign	Ewing
		p-value	
Foliar Treatments	0.9443	0.1932	0.2865
Plant Population	0.3902	<0.0001	0.0004
Plant Pop. *x* Foliar Treat.	0.0061	0.5344	0.1894

*Denotes a significant main effect compared to 80,000 plants acre^{-1}.

Yorkville, LSD $_{\text{Plant Pop. } x \text{ Foliar Treat.}}$ (0.1) = 0.63.

Table 1.6 Effect of foliar treatments and plant population on branches, total pods, pods per branch, and pods per main stem at the R6 growth stage of soybean grown at Champaign, IL in 2019. All values are expressed on a per plant basis.

Foliar Treatments		Branches			Total Pods			Pods Branch^{-1}			Pods Main Stem^{-1}		
AIPGR Application	Foliar Protection	Plant Population, 1000 x plants acre^{-1}											
		80	140	200	80	140	200	80	140	200	80	140	200
None	-	4.0	2.7	2.2	67	46	38	29	13	9	38	33	29
None	Added	4.3	2.6	2.0	68	46	37	31	11	9	37	29	29
V5	-	4.4	2.7	2.1	75	46	38	34	13	7	39	32	29
R3	-	4.9	2.7	2.0	74	45	36	37	14	8	38	32	29
R3	Added	4.2	2.7	2.1	68	40	38	41	15	7	37	32	26
V5 + R3	-	6.1	3.1	2.0	78	47	33	37	13	8	37	32	28
V5 + R3	Added	5.1	2.9	2.4	76	44	39	43	13	10	39	31	30
Average		4.6	2.8*	2.1*	74	45*	37*	36	13*	8*	38	32*	29*
Source of Variation		*p-value*											
Foliar Treatments		0.2820			0.6647			0.3417			0.6814		
Plant Population		<0.0001			<0.0001			<0.0001			0.0003		
Plant Pop. *x* Foliar Treat.		0.8319			0.7261			0.5525			0.8755		

*Denotes a significant main effect compared to 80,000 plants acre^{-1}.

Table 1.7 Effect of foliar treatments and plant population on grain yield and yield components (seed number and seed weight) for soybean grown at Yorkville, IL in 2019. Grain yield is expressed at 13% moisture and seed weight is presented at 0% moisture.

Foliar Treatments		**Plant Population,** plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		Grain Yield, bushels acre^{-1}			
None	-	71.3	73.8	71.6	72.2
None	Added	70.0	71.7	69.2	70.3
V5	-	71.8	70.7	69.7	70.7
R3	-	69.4	70.2	68.9	69.5*
R3	Added	71.9	72.2	71.3	71.8
V5 + R3	-	66.4	67.0	68.3	67.2*
V5 + R3	Added	67.7	72.5	74.8	71.7
Average		69.8	71.1	70.5	
		Seed Number, seeds m^{-2}			
None	-	3123	3193	3074	3130
None	Added	3003	2997	2885	2961*
V5	-	3151	3106	2953	3070
R3	-	3053	3067	2914	3011*
R3	Added	3031	3051	2971	3018*
V5 + R3	-	3948	2922	2906	2925*
V5 + R3	Added	2896	3034	3063	2998*
Average		3029	3053	2966	
		Seed Weight, mg seed^{-1}			
None	-	133	135	136	135
None	Added	136	140	140	139*
V5	-	133	133	138	135
R3	-	133	134	138	135
R3	Added	139	138	140	139*
V5 + R3	-	132	134	137	134
V5 + R3	Added	137	139	143	140*
Average		135	136	139*	

Source of Variation	Grain Yield	Seed Number	Seed Weight
		p-value	
Foliar Treatments	0.0107	0.0025	<0.0001
Plant Population	0.9057	0.6273	0.0117
Plant Pop. *x* Foliar Treat.	0.6009	0.5997	0.8105

*Denotes a significant main effect compared to no treatment or 80,000 plants acre^{-1}.

Table 1.8 Effect of foliar treatments and plant population on grain yield and yield components (seed number and seed weight) for soybean grown at Champaign, IL in 2019. Grain yield is expressed at 13% moisture and seed weight is presented at 0% moisture.

Foliar Treatments		**Plant Population,** plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		Grain Yield, bushels acre^{-1}			
None	-	62.4	62.8	63.6	62.9
None	Added	64.5	65.9	67.0	65.8*
V5	-	62.0	64.0	66.5	64.2
R3	-	59.8	60.4	65.3	61.8
R3	Added	62.4	64.4	64.8	63.9
V5 + R3	-	59.7	65.0	63.4	62.7
V5 + R3	Added	58.7	65.0	66.4	63.4
Average		61.3	63.9	65.3*	
		Seed Number, seeds m^{-2}			
None	-	2758	2856	3127	2914
None	Added	2694	3190	2703	2862
V5	-	2968	3047	2888	2898
R3	-	2967	3027	2795	2945
R3	Added	2718	2817	3243	2880
V5 + R3	-	3011	2615	2938	2855
V5 + R3	Added	2771	2893	3197	2823
Average		2811	2927	2909	
		Seed Weight, mg seed^{-1}			
None	-	127	129	129	128
None	Added	129	131	134	131*
V5	-	126	126	130	127
R3	-	124	125	131	127
R3	Added	129	132	131	130*
V5 + R3	-	124	130	129	128
V5 + R3	Added	127	131	136	131*
Average		126	129	131*	

Source of Variation	Grain Yield	Seed Number	Seed Weight
		p-value	
Foliar Treatments	0.0420	0.8831	<0.0001
Plant Population	0.1846	0.5435	0.0324
Plant Pop. *x* Foliar Treat.	0.3987	0.0038	0.2296

*Denotes a significant main effect compared to no treatment or 80,000 plants acre^{-1}.

Seed Number, LSD $_{\text{Plant Pop. } x \text{ Foliar Treat.}}$ (0.1) = 295.

Table 1.9 Effect of foliar treatments and plant population on grain yield and yield components (seed number and seed weight) for soybean grown at Ewing, IL in 2019. Grain yield is expressed at 13% moisture and seed weight is presented at 0% moisture.

Foliar Treatments		**Plant Population,** plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		Grain Yield, bushels acre^{-1}			
None	-	51.3	52.9	54.1	52.8
None	Added	52.4	54.0	55.9	54.1
V5	-	49.6	52.2	55.9	52.6
R3	-	49.7	52.7	55.4	52.6
R3	Added	51.5	53.3	57.9	54.3
V5 + R3	-	52.0	52.1	56.5	53.5
V5 + R3	Added	52.2	52.0	57.0	53.7
Average		51.3	52.7	56.1*	
		Seed Number, seeds m^{-2}			
None	-	3045	3083	3121	3083
None	Added	3064	3108	3152	3108
V5	-	2939	3024	3181	3048
R3	-	2965	3057	3185	3069
R3	Added	3037	3071	3243	3117
V5 + R3	-	3098	3054	3204	3118
V5 + R3	Added	3019	3094	3197	3103
Average		3024	3070	3183	
		Seed Weight, mg seed^{-1}			
None	-	98	101	101	100
None	Added	100	102	104	102*
V5	-	99	101	103	101
R3	-	98	101	102	100
R3	Added	99	102	105	102*
V5 + R3	-	98	100	103	101
V5 + R3	Added	101	101	104	102*
Average		99	101	103*	

Source of Variation	Grain Yield	Seed Number	Seed Weight
		p-value	
Foliar Treatments	0.2467	0.6548	0.0026
Plant Population	0.0115	0.2315	0.0630
Plant Pop. *x* Foliar Treat.	0.7008	0.8890	0.7525

*Denotes a significant main effect compared to no treatment or 80,000 plants acre^{-1}.

Table 1.10 Effect of plant population and foliar protection on the average number of primary and raceme branches per plant for soybean grown at Champaign, IL in 2020.

Foliar Treatments		Plant Population, plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		branches plant^{-1}			
		Primary			
-	-	5.0	3.7	2.6	3.8
V5	-	4.8	3.2	2.0	3.3
V5	Added	5.2	3.4	2.4	3.7
R3	-	4.4	3.4	2.3	3.4
R3	Added	5.6	3.0	2.4	3.7
V5 + R3	-	4.8	3.1	2.3	3.4
V5 + R3	Added	4.3	3.2	2.6	3.4
Average		4.9	3.3*	2.4*	
		Raceme			
-	-	1.1	0.7	0.4	0.7
V5	-	1.5	0.8	0.6	1.0
V5	Added	0.9	0.5	0.3	0.6
R3	-	1.2	0.5	0.4	0.7
R3	Added	0.9	0.3	0.6	0.6
V5 + R3	-	1.2	0.6	0.6	0.8
V5 + R3	Added	0.9	1.0	0.4	0.8
Average		1.1	0.6*	0.5*	

Source of Variation†	Primary	Raceme
	p-value	
Foliar Treatments	0.6460	0.1146
Plant Population	<0.0001	0.0009
Plant Pop. *x* Foliar Treat.	0.5976	0.5340

† P-value of raceme branches was obtained from transformed data.

*Denotes a significant main effect compared to 80,000 plants acre^{-1}.

Table 1.11 Effect of foliar treatments and plant population on grain yield and yield components (seed number and seed weight) for soybean grown at Yorkville, IL in 2020. Grain yield is expressed at 13% moisture and seed weight is presented at 0% moisture.

Foliar Treatments		Plant Population, plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		Grain Yield, bushels acre^{-1}			
-	-	73.0	73.7	77.3	74.6
V5	-	73.1	74.5	78.3	75.3
V5	Added	72.3	76.3	78.9	75.8
R3	-	70.4	74.2	79.2	74.6
R3	Added	74.4	75.4	78.0	75.9
V5 + R3	-	70.3	74.8	77.4	74.2
V5 + R3	Added	72.0	79.1	79.1	76.8
Average		72.2	75.4*	78.3*	
		Seed Number, seeds m^{-2}			
-	-	3061	3102	3116	3093
V5	-	3084	3106	3136	3109
V5	Added	3002	3113	3150	3088
R3	-	2985	3103	3237	3108
R3	Added	3042	3034	3081	3052
V5 + R3	-	2994	3089	3178	3087
V5 + R3	Added	2972	3170	3154	3099
Average		3012	3097	3150*	
		Seed Weight, mg seed^{-1}			
-	-	140	139	145	141
V5	-	139	140	146	142
V5	Added	141	144	147	144*
R3	-	138	140	143	140
R3	Added	143	146	148	146*
V5 + R3	-	138	144	143	142
V5 + R3	Added	142	146	148	145*
Average		140	143*	146*	

Source of Variation	Grain Yield	Seed Number	Seed Weight
	p-value		
Foliar Treatments	0.4680	0.9488	<0.0001
Plant Population	0.0029	0.0908	0.0003
Plant Pop. *x* Foliar Treat.	0.7574	0.8543	0.1335

*Denotes a significant main effect compared to no treatment or 80,000 plants acre^{-1}.

Table 1.12 Effect of foliar treatments and plant population on grain yield and yield components (seed number and seed weight) for soybean grown at Champaign, IL in 2020. Grain yield is expressed at 13% moisture and seed weight is presented at 0% moisture.

Foliar Treatments		**Plant Population,** plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		Grain Yield, bushels acre^{-1}			
-	-	51.3	61.0	63.5	58.6
V5	-	52.5	63.3	63.8	59.9
V5	Added	56.2	65.1	65.4	62.3*
R3	-	54.3	61.5	62.9	59.6
R3	Added	56.3	65.4	67.6	63.2*
V5 + R3	-	53.4	62.0	65.0	60.2
V5 + R3	Added	57.2	64.1	66.8	62.7*
Average		54.5	63.3*	65.0*	
		Seed Number, seeds m^{-2}			
-	-	2351	2709	2798	2619
V5	-	2354	2764	2849	2655
V5	Added	2492	2794	2893	2726*
R3	-	2445	2722	2810	2659
R3	Added	2465	2876	2952	2764*
V5 + R3	-	2379	2725	2854	2653
V5 + R3	Added	2563	2784	2915	2754*
Average		2436	2768*	2867*	
		Seed Weight, mg seed^{-1}			
-	-	127	132	133	131
V5	-	131	134	131	132
V5	Added	132	137	133	134*
R3	-	130	132	131	131
R3	Added	134	134	134	134*
V5 + R3	-	131	133	133	133*
V5 + R3	Added	131	135	134	133*
Average		131	134	133	

Source of Variation	Grain Yield	Seed Number	Seed Weight
	p-value		
Foliar Treatments	<0.0001	0.0010	0.0414
Plant Population	0.0003	<0.0001	0.3113
Plant Pop. *x* Foliar Treat.	0.8122	0.7780	0.5950

*Denotes a significant main effect compared to no treatment or 80,000 plants acre^{-1}.

Table 1.13 Effect of foliar treatments and plant population on grain yield and yield components (seed number and seed weight) for soybean grown at Nashville, IL in 2020. Grain yield is expressed at 13% moisture and seed weight is presented at 0% moisture.

Foliar Treatments		Plant Population, plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		Grain Yield, bushels acre^{-1}			
-	-	55.3	57.5	57.8	56.9
V5	-	55.2	58.5	57.8	57.2
V5	Added	55.1	55.5	58.8	56.5
R3	-	55.4	57.7	56.0	56.3
R3	Added	56.0	56.7	56.6	56.4
V5 + R3	-	55.8	55.9	56.3	56.0
V5 + R3	Added	56.8	58.7	57.3	57.2
Average		55.7	57.2	57.2	
		Seed Number, seeds m^{-2}			
-	-	2575	2616	2637	2610
V5	-	2645	2697	2593	2645
V5	Added	2539	2450	2548	2512*
R3	-	2559	2550	2542	2550
R3	Added	2538	2470	2445	2484*
V5 + R3	-	2641	2562	2486	2563
V5 + R3	Added	2558	2641	2501	2667
Average		2579	2570	2536	
		Seed Weight, mg seed^{-1}			
-	-	126	129	128	128
V5	-	122	127	131	126
V5	Added	127	133	135	132*
R3	-	127	132	129	129*
R3	Added	129	133	136	133*
V5 + R3	-	124	128	133	128
V5 + R3	Added	130	130	134	132*
Average		126	130*	132*	

Source of Variation	Grain Yield	Seed Number	Seed Weight
		p-value	
Foliar Treatments	0.5981	0.0077	<0.0001
Plant Population	0.5749	0.6227	0.0870
Plant Pop. *x* Foliar Treat.	0.5001	0.3890	0.0226

*Denotes a significant main effect compared to no treatment or 80,000 plants acre^{-1}.

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CHAPTER 2. ENHANCING NITROGEN UPTAKE AND CORN PRODUCTIVITY WITH *AZOSPIRILLUM BRASILENSE*

INTRODUCTION

There is much of concern about the ability to produce the calories for the projected world population of 9.7 billion people by 2050 (United Nations, 2019). This estimate is 2 billion more than there are currently living on Earth, with the largest increases projected in developing third world countries. By itself, this change is not concerning, but a consequence of an upward movement of people to higher social classes is an increase in the consumption of meat (Whitnall and Pitts, 2020). As livestock are not efficient at converting calories from grain into protein, there is a parallel need for increased grain yields to feed greater numbers of livestock. Compounding the challenge of increases in population and meat consumption is the loss of land for agricultural production (Alexandratos and Bruinsma, 2012). Agricultural production needs to be more efficient, to meet these challenges because farmers cannot simply increase inputs on fewer acres due to the risk of environmental damage. A highly efficient grain crop would help ameliorate the land-loss problem, and corn (*Zea mays*, L.) has the potential to produce twice the grain yield of other cereal crops (Tollenaar and Lee, 2002; Abebe and Feyisa, 2017), making it a good option as one of the key crops to supply the world with calories. However, to achieve high grain yields, the corn crop must be intensely managed using multiple agronomic factors, including fertility, hybrid, planting population, and foliar protection (Ruffo et al., 2015).

Fertility is often the most limiting factor to corn grain yield, and more specifically the nutrient nitrogen (N) due to the high levels of N accumulated by the crop (Below, 2002). For a 230 bushel per acre yield, the crop must accumulate 256 lbs of N acre (Bender et al., 2013). This high N requirement is because N is a key component in essential plant organic compounds such as proteins, nucleic acids, chlorophyll, and growth regulators (Fernández et al., 2009), and is important for establishing and maintaining the photosynthetic apparatus and reproductive sink capacity (Below, 2002). Therefore, N is the most abundant element in the plant that is not obtained from water or CO_2. In the hope of achieving high grain yields, farmers tend to over-fertilize with N to assure that nutrient supply is not a limiting factor. While greater application of N fertilizer is a feasible solution to low yields in many developing countries, consideration must be given to the availability of inexpensive fertilizers, as fertilizer cost is a major deterrent in developing countries.

In addition to the cost, N fertilizers also have the potential for environmental damage depending on the rate, source, placement, and time of application. Nitrogen as nitrate is mobile in the soil and if nitrate accumulates in the groundwater used for wells it can cause methemoglobinemia, which can be fatal particularly to infants or young animals (Majumdar, 2003). Alternatively, nitrate can end up in streams or rivers where it can cause eutrophication (Shannon and Brezonik, 1972). Soil nitrate is also susceptible to another mechanism for loss from denitrification as a gas back to the atmosphere. Denitrification is the result of soil microbes using the nitrate molecule as a terminal electron acceptor in their respiration when in an anaerobic environment.

Nitrogen can be taken up by the plant in two forms: ammonium (NH_4^+) and NO_3^-. Nitrate is the most common form of plant uptake, as ammonium is less available in the soil solution due to: 1) the rapid conversion of ammonium to nitrate by soil microbes (nitrification); 2) soil microbes using NH_4^+ for growth (immobilization); and, 3) the positively charged NH_4^+ cation being held by negatively charged soil colloids (Scharf, 2015a). The rates of these processes are based on the water saturation and the temperature of the soil. However, even when conditions are ideal for increasing the plant-available inorganic N from the organic pool, the corn crop can still be limited by the lack of N, because soil mineralization usually cannot supply all the N that a high yielding corn crop needs. In addition to being the main form of N that is taken up by the plant nitrate can also be stored in vacuoles of the plant for later assimilation (Below, 2002; Scharf, 2015b). Nitrate must be reduced to NH_4^+ and assimilated into amino acids and eventually proteins to be used by the plant. The lesser available N form, ammonium, also can absorbed by plants, but it must be assimilated immediately into amino acids in the root to prevent ammonia (NH_3) toxicity.

The search for environmentally-friendly ways to supply N to growing crops without increasing the number of fertilizer N applications, leads to the possibility of using microbes to supplement the supply N. With the growing interest in biostimulants, the United States Congress found it was necessary to define biostimulants in the 2018 Farm Bill as "a substance or micro-organism that, when applied to seeds, plants, or the rhizosphere, stimulates natural processes to enhance or benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, or crop quality and yield" (Agriculture Improvement Act, 2018). Nitrogen-fixing bacteria are classified as biostimulants, and their ability to fix atmospheric N maybe a way to increase plant N supply and enhance grain yield. *Azospirillum brasilense* is an example of a free-living nitrogen-fixing bacteria that has the potential to provide additional plant-available N, while also producing plant-growth stimulating compounds like auxin (Okon et al., 1994) (Sumner, 1990) (Tien et al., 1979).

A common way to apply, biostimulants, including nitrogen-fixing-bacteria is in-furrow where the close placement to the seed can increases nutrient availability and plant uptake. Because *A. brasilense* is typically applied in-furrow at planting this practice avoids an additional equipment trip through the field, thereby eliminating in-season application cost and preventing field compaction from application equipment.

There are critical periods in corn growth that influence yield potential and grain yield (Abendroth et al., 2011; Fageria et al., 2006), and these stages are relevant when it comes to enhancing the plant available N supply with N-fixing bacteria. The first growth stage that affects yield potential is V5 to V6, when the number of kernels around an ear is initiated. Similarly, the number of kernels in a row on an ear and as such the total kernel potential is determined between V6 and V12. The final stage for kernel number determination is VT to R1, or during pollination, as plant stress at these stages can lead to poor pollination decreasing kernel number and in turn grain yield (Setter et al., 2001). Kernel weight is influenced by the duration of grain-fill during reproductive stages (R3-R5), as more than half of the kernel weight is accumulated during the dough and dent stages (Wilhelm et al., 1999). However, the direct effects of the number or the average weights of the kernels on grain yield is not always clear as sometimes one component can partially compensate for another in what is known as yield component compensation (Adams, 1967).

The objective of this research was to determine if an in-furrow application of *A. brasilense* can increase the N supply and the productivity of corn. Multiple N rates were examined with and without *A. brasilense* to determine the N replacement value as well as the N rate that gives maximal N use and yield. The research was conducted at three sites in the state of Illinois during 2019 and 2020. Knowing the right environment and the N rate to be applying *A. brasilense* will provide farmers with data-backed recommendations, and could in turn increase the efficiency of fertilizer N use and productivity of corn.

MATERIALS AND METHODS

Field Characteristics

The trial was implemented in the 2019 and 2020 growing seasons at three locations across the state of Illinois. In 2019, this study was conducted at the Crop Science Research and Education Center (CSREC) located at the University of Illinois Urbana-Champaign and two off-site locations: at Yorkville, IL, in the northern part of the state and Ewing, IL, in the southern part of the state. Soil types differed between locations with the Yorkville and Champaign locations consisting of a Drummer silty clay loam soil type, and the Ewing location consisting of a Cisne silt loam. In 2020, the study was implemented at Yorkville and Champaign, as well as an alternate southern location at Nashville, IL. The soil types in 2019 were Drummer silty clay loam at Yorkville and Champaign, and a Hoyleton silt loam at Nashville. Pre-plant soil samples (0-6 in deep) were obtained from plot areas prior to planting and analyzed (A & L Great Lakes Laboratories, Fort Wayne, IN) to confirm soil fertility levels.

Pesticide Applications

In both years, all plots received an in-furrow soil insecticide application of tefluthrin ([2,3,5,6-tetrafluoro-4-methylphenyl] methyl-[1α,3α]-[Z]-[±]-3-[2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethylcyclopropanecarboxylate), known as Force 3G (Syngenta, Basel, Switzerland) at a rate of 4 oz acre^{-1} in 2019, and tefluthrin known as Force 6.5G (Syngenta, Basel, Switzerland) at a rate of 5 lbs acre^{-1} in 2020.

In 2019, all locations were maintained weed-free in part with a pre-emergence herbicide application of acetochlor (2-chloro-2'-methyl-6'-ethyl-N(ethoxymethyl)acetanilide) + atrazine (1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) known as Breakfree ATZ (Corteva Agriscience, Wilmington, DE) at a rate of 2 qt acre^{-1} at Yorkville, IL; bicyclopyrone (bicyclo[3.2.1]oct-3-en-2-one, 4-hydroxy-3-[[2-[(2-methoxyethoxy)methyl]-6-[trifluoromethyl]-3-pyridinyl]carbonyl]) + mesotrione (2-[4-[methylsulfonyl]-2-nitrobenzoyl] cyclohexane-1,3-dione) + S-metolachlor (2-chloro-N-[2-ethyl-6-methylphenyl]-N-[2-methoxy-1-methylethyl] acetamide) + atrazine known as Acuron (Syngenta, Basel, Switzerland) at a rate of 96 oz acre^{-1} at Champaign, IL; and Acuron at a rate of 64 oz acre^{-1} at Ewing, IL.

In 2019, in-season weed control at Yorkville was applied at the V6 growth stage with S-metolachlor + glyphosate (N-phosphonomethyl glycine, in the form of a potassium salt)+ mesotrione, known as Halex GT (Syngenta, Basel, Switzerland) at a rate of 57 oz acre^{-1}; sodium salt of diflufenzopyr [2-(1-[([3,5-difluorophenylamino]carbonyl)-hydrazono]ethyl)-3-pyridinecarboxylic acid, sodium salt] + sodium salt of dicamba (3,6-dichloro-2-methoxybenzoic acid, sodium salt) also known as Status (BASF Corporation, Ludwigshafen, Germany) at 4 oz acre^{-1}; AAtrex 4L (Syngenta, Basel, Switzerland) at a rate of 32 oz acre^{-1}; glyphosate (Glyphosate, N-(phosphonomethyl)glycine, in the form of its potassium salt) RoundUp PowerMax at a rate of 13 oz acre^{-1}; Alkyl polyethoxy ethers, ethoxylated and soybean oil derivatives known as FS AquaSupreme (FS Growmark, Bloomington, IL) surfactant at a rate of 0.1 gal acre^{-1}; and ammonium sulfate (AMS; 21-0-0-24S) at a rate of 0.2 gal acre^{-1}. In-season weed control at Champaign was performed at the V5 growth stage consisting of topramezone ([3-(4,5-dihyrdo-isoxazolyl)-2-methyl-4-(methylsulfonyl) phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone) known as Armezon (BASF, Ludwigshafen, Germany) at 0.75 oz acre^{-1}, AAtrex at a rate of 32 oz acre^{-1}, Status at 4 oz acre^{-1}, RoundUp PowerMax at 32 oz acre^{-1}, and AMS at a rate of 0.2 gal acre^{-1}. At Ewing, the in-season weed control was applied at the V6 growth stage containing tembotrione (2-[2-chloro-4-[methylsulfonyl]-3-[(2,2,2-trifluoroethoxy) methyl]benzoyl]-1,3-cyclohexanedione) known as Laudis (Bayer, St. Louis, MO) at a rate of 3 oz acre^{-1}; AAtrex 4L at a rate of 32 oz acre^{-1}; RoundUp PowerMax at a rate of 32 oz acre^{-1}; and AMS at a rate of 0.2 gal acre^{-1}.

In 2020, Yorkville and Nashville were maintained weed-free partially with a pre-emergence herbicide application of atrazine known as Infantry 4L (FS Growmark, Bloomington, IL) at rate of 20 oz acre^{-1}, and Acuron at a rate of 96 oz acre^{-1} in Yorkville and at a rate of 72 oz acre^{-1} in Nashville. The pre-emergence herbicide application in Champaign consisted of Infantry 4L at rate of 32 oz acre^{-1}, and acetochlor + mesotrione + clopyralid MEA salt (3,6-dichloropyridinecarboxylic acid, monoethanolamine salt) known as Resicore (Corteva Agriscience, Wilmington, DE) at a rate of 88 oz acre^{-1}.

In-season weed control at Yorkville and Champaign in 2020 was applied at the V5 growth stage with glyphosate (N-(phosphonomethyl) glycine, in the form of its potassium salt) known as RoundUp WeatherMaxx at rate of 32 oz acre^{-1}, Infantry 4L at rate of 3 oz acre^{-1}, FS AquaSupreme surfactant at a rate of 0.1 gal acre^{-1}, AMS at a rate of 0.2 gal acre^{-1}, diglycolamine salt (3,6-dichloro-o-anisic acid) known as DiFlexx (Bayer, St. Louis, MO) at a rate of 16 oz acre^{-1}, and Laudis at a rate of 3 oz acre^{-1}. In-season weed control in Nashville occurred at the V6 growth stage consisting of RoundUp WeatherMaxx at rate of 32 oz acre^{-1}, Infantry 4L at rate of 3 oz acre^{-1}, FS AquaSupreme surfactant at a rate of 0.1 gal acre^{-1}, AMS at a rate of 0.2 gal acre^{-1}, DiFlexx at a rate of 16 oz acre^{-1}, and Laudis at a rate of 3 oz acre^{-1}.

Agronomic Management

Soybean was the previous crop and conventional tillage was used at all sites in both years. A nitrogen-responsive hybrid was grown in 30-inch row spacing at a planting population of 36,000 plants acre^{-1}, Golden Harvest 10L16 (Syngenta, Basel, Switzerland) with a 110-day relative maturity. This hybrid was sown with a precision plot planter, SeedPro 360 (ALMACO, Nevada, IA) on 8 June 2019 at Yorkville, 2 June 2019 at Champaign, and 4 June 2019 at Ewing. In the following year, plots were planted on 5 June, 1 June, and 7 June 2020 at Yorkville, Champaign, and Nashville, respectively.

Treatment Applications

Applications were designed to evaluate a free-living nitrogen fixing bacteria, *Azospirillum brasilense,* known as GRAP NODa (Agrocete, Cara-Cara, Paraná, Brazil), for its role in nitrogen use and productivity of corn. In both years, this product was supplied to half of the plots in-furrow at planting with a planter-attached liquid starter applicator system (Surefire Ag Systems, Atwood, KS) and at a total volume rate of 8 gal acre^{-1} with water as a carrier. *Azospirillum brasilense* was evaluated across five preplant N rates (0, 50, 100, 150, and 200 lbs N acre^{-1}) that were broadcast pre-plant as urea (46-0-0) using a hand-held spreader and incorporated into the soil before planting. All treatments of *A. brasilense* were applied at a rate of 13.7 oz acre^{-1}.

Experimental Design and Statistical Analysis

In 2019 and 2020 treatments were arranged in a randomized complete block design with six replications and ten treatments for a total of 60 plots at each location (grand total of 360 plots). Each plot was four rows wide and 37.5 ft in length with 30 in row spacing. Statistical analysis was conducted using PROC MIXED in SAS (version 9.4; SAS Institute, Cary, NC). *Azospirillum brasilense* treatment and N fertilizer rate were considered fixed effects, with location as a random factor in the model. Significance was declared at $P \leq 0.10$. PROC GLM of SAS was utilized to conduct the Brown-Forsythe test of the Levene test for homogeneity of variance on the errors and significance was declared at $P \leq 0.05$. PROC UNIVARIATE of SAS was used to determine possible outliers and assess the normality of the errors, with significance declared at $P \leq 0.05$. In addition to the Shaprio-Wilk test, QQ plots and histograms were studied to determine normality of the errors, when the Shapiro-Wilk tests were significant. With homogeneity of variance and normality assumptions met, the locations were analyzed separately by year due to differing responses caused by the environment.

Measured Parameters

At Champaign in 2019, total shoot biomass accumulation was measured at the R6 (physiological maturity) growth stage, but in 2020 at all three locations, total shoot biomass sampling was measured at both the V8 (eight leaves) and R6 growth stages. Total shoot biomass sampling consisted of manually cutting two representative plants of the plot at the soil surface from each of the center two rows of each plot (four plants total). The V8 sampled plants were placed into a forced air oven at 167 °F until reaching 0% moisture, and then weighed to obtain the dry biomass accumulation. The plants at R6 were partitioned into grain and stover (including husk) components, and biomass accumulation was determined by weighing the total fresh stover then processing it through a chipper (BC600XL, Vermeer Corporation, Pella, IA) to obtain representative stover subsamples. The stover subsamples were immediately weighed to determine the aliquot fresh weight, and then weighed again after drying to 0% moisture in a forced air oven at 167 °F to determine subsample aliquot dry weight and calculate total dry biomass accumulation. Corn ears with husks removed were dried, the grain was removed using a corn sheller (AEC Group, St Charles, IA) and analyzed for moisture content using a moisture tester (Dickey John, GSF, Ankeny, IA). Cob weight was obtained by the difference between ear and grain weights, and dry stover and cob weights were summed to calculate the overall R6 stover biomass. Subsamples of the aboveground stover from the V8 and R6 sampling were ground to 2 mm particle sizes using a Wiley Mill (Thomas Scientific, Swedesboro, NJ). An approximately 50 mg subsample of the ground tissue was randomly selected and analyzed for N using a combustion-based analyzer

(EA1112, CE Elantech, Lakewood, NJ). Plots were sampled for V8 biomass on: 7 July at Yorkville, 3 July at Champaign, and 6 July at Nashville in 2020. Plots were sampled for R6 biomass on: 16 October 2019 at Champaign, 13 October 2020 at Yorkville, 25 September 2020 at Champaign, and 21 September 2020 at Nashville.

Following physiological maturity in both years, the center two rows of each plot were harvested with a combine (SPC40 in 2019 and an R1 in 2020, ALMACO, Nevada, IA) to determine grain yield and harvest moisture, and the yield subsequently standardized to bushels per acre at 15.5% moisture. The harvest dates in 2019 were 20 November, 22 October, and 14 October 2019, at Yorkville, Champaign, and Ewing, respectively. In the second year of this study, the harvest dates were 1 November, 20 October, and 6 October 2020, at Yorkville, Champaign, and Nashville, respectively. The combine also collected subsamples of the harvested grain that were evaluated for grain quality (protein, oil, and starch concentrations at 0% grain moisture) by utilizing near-infrared transmittance spectroscopy (NIT) (Infratec 1241 Grain Analyzer, Foss, Eden Prairie, MN). Average kernel weights were estimated based on a representative subsample of 300 kernels and adjusted to 0 % moisture. Kernel number on a per-acre basis was obtained from dividing total grain weight by the average kernel weight.

Grain N concentration was calculated algebraically by dividing grain protein concentration obtained by NIT by the constant 6.25 (Jones, 1941). Following calculation of the grain N concentration, the final grain yield was used to determine total grain N content. Total N uptake was calculated by adding grain nitrogen content and the stover N content that was determined by the combustion method. Harvest index was then also calculated by dividing the total weight of grain from plants sampled for the total shoot biomass divided by the total weight of stover from same plants sampled for the total shoot biomass.

RESULTS AND DISCUSSION

Soil Characteristics

Preplant composite soil test values varied across the locations (Table 2.1). In both years, there was increasing inherent soil fertility from the southern (Ewing and Nashville) to the northern (Yorkville) field sites in Illinois. Notably, native soil organic matter and CEC levels tended to increase moving from southern to northern Illinois. Also, in both years, Yorkville soils contained the highest inherent soil fertility levels in combination with a slightly lower pH (Table 2.1).

Weather

Due to excessive spring precipitation at all locations in 2019, and/or excessive precipitation and untimely rains at all sites in 2020 (Table 2.2), these trials were planted a month later than normal, leading to a shorter than optimal growing season. The Yorkville location in 2019 experienced the most rainfall, receiving 16.8 inches more precipitation than the 30-year average throughout the season (Table 2.2). Overall in 2019, the Champaign and Ewing locations had excess precipitation early in the year but were warmer and drier than the 30-year average July through September. Throughout the 2020 growing season, seasonal precipitation and average temperatures were close to the 30-year averages at Yorkville and Champaign, but both sites experienced unique weather challenges that negatively affected yield. At Champaign, a hail storm on July 11 caused up to 30 % leaf defoliation, and at Yorkville a derecho wind storm caused some green snap and stem lodging. Both the Champaign and the Yorkville sites also experienced a lack of precipitation during the month of August that negatively affected kernel-filling (Table 2.2). In 2019, Champaign was dry in August during tasseling and pollination, while Ewing was much drier than average in September during grain fill (Table 2.2). In 2020, the Nashville location received almost 10 inches more seasonal precipitation than the 30-year average, with the majority of this precipitation occurring in July and early August (Table 2.2). The excess precipitation in July and early August, along with above-average temperatures in July led to a higher than normal incidence of leaf disease (Southern Rust) at the Nashville site.

Plant Biomass and N Accumulation

At all locations in 2020, vegetative biomass accumulation at the V8 growth stage increased with N application and in many instances further increased with *A. brasilense* treatment (Table 2.3). At Yorkville and Champaign, the main effect of *A. brasilense* was statistically significant for total biomass accumulation at V8, while at Nashville there was a significant *A. brasilense* by N rate interaction. At Nashville, *A. brasilense*-treated plants were 7 % larger at the 0 and 100 lbs acre^{-1} N rates (Table 2.3). Averaged over all N rates, *A. brasilense*-treated plants were 7 % larger at Yorkville and 5 % larger at Champaign, although at individual N rates there were instances where *A. brasilense*-treated plants were substantially larger (i.e 14 % larger with 150 lbs N acre^{-1} at Champaign and 15 % larger with 100 lbs N acre^{-1} in Yorkville), and these differences were visually apparent (Figure 2.1). The bigger plants can be attributed to ideal early season weather and the additional N supplied by *A. brasilense* that in combination allowed for greater growth, setting a high grain yield potential. Similar findings have been reported by Zeffa et al. in 2019 who showed that the addition of *A. brasilense* increased early season growth by 13.8 % on average at the low N rate. Associated with the increase in vegetative plant dry weight was additional N accumulation, either as a function of increasing rates of N and at some sites from *A. brasilense* application (Table 2.3). At Champaign, the main effect of *A. brasilense* on V8 plant N accumulation was statistically significant, while the interaction of *A. brasilense* by N rate was significant at both Champaign and Yorkville. At Yorkville, the *A. brasilense* by N rate interaction resulted from a 17 % increase in plant N accumulation from *A. brasilense* treatment at the highest rate of applied N (200 lbs N acre^{-1}), but no effect at the other N rates (Table 2.3). At Champaign, *A. brasilense* treatment increased plant N accumulation by 11 % when no fertilizer N was applied and by 18 % at the 150 lb N acre^{-1} rate. Due in part to those large increases, NDVI measurements at the V8 growth stage at Champaign revealed a significant main effect of *A. brasilense*. Collectively, this data shows an enhancement in vegetative growth from the in-furrow application of *A. brasilense*, which in some cases was associated with additional accumulation of plant N (Tables 2.3). The greater early season growth from *A. brasilense* has been reported to be associated with root growth which, can be attributed to the auxin-producing capability of *A. brasilense* in combination with its ability to biologically fix atmospheric N (Zeffa et al., 2019; Lin et al., 1983; Albrecht et al., 1981; and Sumner, 1990). Additionally, this larger root mass may have aided the N-fixing abilities of *A. brasilense* in making the plants larger and darker green as noted by the larger NDVI values, and enhanced the uptake of immobile nutrients like phosphorus (Table 2.3). This data shows that, *A. brasilense* fostered better early-season plant growth, especially in higher-yielding environments (Yorkville and Champaign).

Similar to the V8 growth stage data, the application of N fertilizer increased total aboveground biomass accumulation at physical maturity (R6 growth stage) (Tables 2.4, 2.5, and 2.6). However, in contrast to the V8 growth stage, there was no effect of *A. brasilense* treatment on R6 biomass accumulation at any site, and in some cases, there was a tendency for *A. brasilense*-treated plants to have less biomass accumulated at R6. The tendency for smaller plants at R6 from *A. brasilense* treatment was especially apparent at the higher rates of N application (150 and 200 lbs N acre^{-1}). This result may be due to adequate N resulting initially in larger plants that were more adversely affected by the wind damage at Yorkville, the lack of precipitation during the month of pollination (July) at Champaign in 2019, the hail damage at Champaign in 2020, and the high instance of leaf disease in Nashville. Similar to dry weight, most of the enhancement in plant N accumulation due to *A. brasilense* observed at V8 (Table 2.3) was no longer present at R6, except at Champaign in 2020, where supplying *A. brasilense* led to modest, but statistically significant, greater plant N uptakes (Table 2.5). At Yorkville, *A. brasilense*-treated plants nominally had greater plant N accumulation at the 50 lbs N acre^{-1} rate, while at all locations except for Nashville in 2020, *A. brasilense*-treated plants had nominally greater total N accumulation at the 200 lb N acre^{-1} rate (Tables 2.4, 2.5, and 2.6). Albrecht et al. (1981) observed similar results that at physiological maturity, shoot total N uptake typically increased with the application of *A. brasilense*.

Grain Yield and Yield Components

At Yorkville, the corn plants responded to the addition of N fertilizer, with the maximum yields achieved with 150 lbs N acre^{-1} in 2019 and 100 lbs N acre^{-1} in 2020 (Table 2.7). However, supplying *A. brasilense* did not significantly increase yield either year, or at any N rate, but there were numerical increases in grain yield with the addition of *A. brasilense*; 2 bushel acre^{-1} at 0 lbs N acre^{-1} in 2019, 4 bushel acre^{-1} at 100 lbs N acre^{-1} in 2019, 2 bushel acre^{-1} at 50 lbs N acre^{-1} in 2020, and 4 bushel acre^{-1} at 200 lbs N acre^{-1} in 2020. Conversely, at the 100 lbs N acre^{-1} rate in 2020, a 9 bushel acre^{-1} grain yield decrease was observed from supplying *A. brasilense*. Unlike grain yield, kernel number was not always significantly affected by N as seen in 2020 and can be attributed to N fertilization beyond 50 lbs N acre^{-1} having little impact on the kernel number, likely because the 5.3% organic matter soil at Yorkville (Table 2.1) mineralized enough N to compensate for any nitrogen deficiency (Azam et al., 1993). Conversely, in 2019, kernel numbers were significantly affected by the N rate. Also in 2019, kernel number significantly interacted with N rate and *A. brasilense* application, primarily that *A. brasilense* application resulted in an increase in kernel number only at the 100 lb N acre^{-1} rate at Yorkville (Table 2.7). In both years, the N rate influenced final kernel weight at Yorkville, as there was a strong tendency to increase kernel weight with increases in the preplant N applied. In 2020, kernel weight was not significantly affected by *A. brasilense* supply, but there was a tendency for the addition of *A. brasilense* to decrease the kernel weight. The only significant effect of *A. brasilense* application on grain quality at Yorkville was the tendency to increase grain protein concentration at the higher N rates in 2020 (Table 2.7). Nitrogen rate, however, always significantly affected final grain quality at Yorkville, except for grain starch concentration in 2019.

At Champaign, grain yield, kernel number, and kernel weight were significantly affected by the addition of N fertilizer with the maximum yields achieved using 200 lbs N acre^{-1} in 2019 and 150 lbs N acre^{-1} in 2020 (Table 2.8). The addition of *A. brasilense* in combination with 0, 50, or 200 lbs N acre^{-1} in 2019 led to nominal yield increases, ranging from 2 to 9 bushel acre^{-1}. In 2020, yield increases from the addition of *A. brasilense* ranged from 1 to 7 bushel acre^{-1} in combination with up to 150 lbs N acre^{-1} in 2020 at Champaign. However, similar to the Yorkville location in 2020, supplying *A. brasilense* at the moderate N rates, 100 and 150 lbs N acre^{-1}, resulted in non-significant yield decreases of 22 bushel acre^{-1} and 17 bushel acre^{-1}, respectively in 2019. Dobbelaere et al. (2001) showed that *A. brasilense* was more effective when applied on maize and sorghum with N fertilization of 0 to 80 lbs N acre^{-1} as opposed to 89 to 133 lbs N acre^{-1}. This data supports those findings that supplying *A. brasilense* is more effective in increasing yields in combination with low to moderate N rates than with high N. In 2019, there was an interactive effect of N rate and *A. brasilense* application on kernel numbers, due to *A. brasilense* applications increasing kernel number at the low N rates, but decreasing kernel numbers at the higher N rates (Table 2.8). Kernel weights were not significantly changed by *A. brasilense* treatments, but there was a tendency for kernel weight to be the greatest at the highest N rates (150 and 200 lbs N acre^{-1}) and when in combination with *A. brasilense.* In 2019 at Champaign, the only significant difference detected in grain quality due to the addition of *A. brasilense* was that at the 100 and 150 lbs N acre^{-1} rates, the grain starch concentrations were significantly increased. Similar to previous findings (Tsai et al., 1992), N rate caused significant differences in the grain protein concentration in both years and tended to increase with successive increases in the N rate (Table 2.8).

At the southern Illinois locations, Ewing in 2019 and Nashville in 2020, N was a significant source of variation for grain yield, kernel number, kernel weight similar to Yorkville and Champaign in 2019 and 2020. Despite the responsiveness to fertilizer N at all sites, *A. brasilense* did not significantly increase grain yield but did generate nominal grain yield increases at the low N rates (0 and 50 lbs N acre^{-1}) at Ewing. At the moderate N rates (100 and 150 lbs N acre^{-1}), adverse responses were observed compared to Champaign in 2019 and Yorkville in 2020. Interestingly, yield responses to the treatments at Ewing in 2019 was most similar to Champaign in 2019, and yield responses at Nashville in 2020 was most similar to Yorkville in 2020. In both years, the yield components (kernel number and kernel weight) were significantly affected by the N rate, as the increases in the N rate tended to increase both yield components (Table 2.9). The southern location of Ewing in 2019 generated significant differences in grain protein concentration with the applications of either N rate or *A. brasilense*. In 2020, the N rate application significantly affected both the grain protein and starch concentrations at Nashville.

CONCLUSION

The variable results in growth and yield of corn in response to both *A. brasilens*e and to N supply at the different locations and years demonstrates the large role of the environment in the use of biostimulants to improve nutrient use and to increase productivity. However, despite this variability, there were some positive vegetative growth responses to *A. brasilense* application, especially at the higher yielding sites (Yorkville and Champaign), and especially at the lower and higher rates of N, and these growth increases trended towards greater kernel production and higher yields (in the 2 to 12 bushel per acre range). Conversely, at some sites and years there were also some yield decreases (3 to 22 bushels per acre) from *A. brasilense* when used with the intermediate N rates. Collectively, this study demonstrates the interactive nature of living microbes and N supply, and shows the need for additional research on how to best use living microbes like *A. brasilense* to improve N use in corn production.

TABLES AND FIGURE

Table 2.1 Pre-plant soil properties and Mehlich 3-extraction-based mineral test results for *Azospirillum brasilense* trials conducted at Yorkville, Champaign, Ewing, and Nashville, Illinois in 2019 and 2020.

Year	Location	OM	CEC	pH	P	K	Ca	Mg	S	Zn	Mn	Fe	Cu	B
		%	meq/100g	unit	ppm									
2019	Yorkville	7.0	31.2	6.0	35	97	3913	793	13	3	11	142	3	0.7
	Champaign	3.3	26.6	6.4	34	120	3010	581	7	1	19	123	3	0.7
	Ewing	2.5	11.3	7.3	49	70	2051	99	10	1	131	194	1	0.2
2020	Yorkville	5.3	23.8	6.3	168	161	2847	669	13	9	25	200	5	0.8
	Champaign	4.1	21.3	6.6	22	91	2892	648	9	1	25	100	2	0.7
	Nashville	2.1	12.0	6.7	11	81	1829	169	10	3	114	145	1	0.1

Table 2.2 Monthly precipitation and temperature during the production season at Yorkville, Champaign, Ewing, and Nashville, IL in 2019 and 2020 compared to the 30-year average. Values obtained from the Illinois State Water Survey.

	Year							
	2019				2020			
	Precipitation		Temperature		Precipitation		Temperature	
Month	Season	30-Year Average	Season	30-Year Average	Season	30-Year Average	Season	30-Year Average
	—— inches ——		—— °F ——		—— inches ——		—— °F ——	
				Yorkville				
April	4.8	3.9	48	50	3.6	3.0	46	49
May	8.4	3.6	58	61	6.1	3.8	58	60
June	2.6	3.8	69	70	3.3	3.8	72	70
July	2.8	3.2	75	72	4.4	3.2	74	72
August†	4.4	3.4	69	70	0.9	3.4	70	70
September	12.0	2.7	67	63	5.1	3.0	61	63
October	5.1	2.7	48	52	2.2	2.8	47	51
Total/Average	40.1	23.3	62	63	25.6	23.0	58	62
				Champaign				
April	5.3	3.7	53	53	5.3	3.7	50	53
May	5.2	4.7	64	63	4.7	4.7	61	63
June	3.7	4.4	72	73	5.8	4.4	74	72
July‡	2.3	4.2	77	75	4.6	4.1	77	75
August	2.1	3.4	74	74	1.3	3.4	73	74
September	3.3	3.1	72	67	2.9	3.1	65	67
October	5.0	3.2	54	55	2.4	3.3	52	55
Total/Average	26.9	26.7	67	66	27.0	26.7	65	66
		Ewing				Nashville		
April	7.1	4.8	58	58	4.7	4.4	54	56
May	7.0	4.7	67	67	4.3	4.9	64	66
June	3.5	4.0	73	75	4.0	3.9	77	74
July	2.1	3.6	79	78	9.1	3.3	80	77
August	2.2	3.1	76	76	7.5	3.3	75	75
September	0.3	3.5	75	69	0.6	2.9	68	67
October	8.0	3.5	57	58	5.1	2.9	55	57
Total/Average	30.2	27.2	69	69	35.3	25.6	68	67

† August 10th, 2020 recorded a derecho with winds ranging 70 -126 mph.

‡ July 11th, 2020 recorded 0.75 – 1.5 inch sized hail.

Table 2.3 Effect of N and *Azospirillum brasilense* addition on N accumulation, dry biomass, and NDVI at the V8 growth stage of corn at three locations in Illinois in 2020.

Preplant N Rate	Biomass		N Accumulation		NDVI	
	Added *Azospirillum brasilense*†					
	-	+	-	+	-	+
lbs N/acre	grams/plant		lbs N/acre		NDVI unit	
			Yorkville			
0	15.5	15.1	38.4	38.3	0.54	0.53
50	16.9	19.0*	46.0	46.0	0.52	0.53
100	16.9	19.5*	47.0	46.1	0.56	0.54
150	17.9	17.7	50.1	48.0	0.54	0.55
200	17.1	19.1*	47.0	55.2	0.53	0.53
Average	16.7	18.1*	45.7	46.7	0.54	0.54
Source of Variation			***p-value***			
N	0.0037		<0.0001		0.5939	
(*A.b.*)	0.0248		0.4140		0.7718	
N *x* (*A.b.*)	0.2294		0.0894		0.6807	
			Champaign			
0	11.2	12.7*	24.4	27.1*	0.55	0.56*
50	14.1	14.9*	37.3	38.3	0.58	0.59*
100	14.9	15.1	36.8	37.2	0.60	0.60
150	13.1	15.0*	37.8	44.6*	0.57	0.60*
200	14.5	13.9	42.2*	40.0	0.57	0.57
Average	13.6	14.3*	35.7	37.4*	0.57	0.59*
Source of Variation			***p-value***			
N	0.0065		0.0006		0.0003	
(*A.b.*)	0.0750		0.0951		0.0598	
N *x* (*A.b.*)	0.2961		0.0956		0.3143	
			Nashville			
0	17.4	18.7	35.3	35.5	-	
50	17.7	18.0	39.7	40.4	-	
100	18.6	19.9	45.7	43.8	-	
150	21.9	21.1	57.1	56.9	-	
200	23.0	19.3	58.1	51.0	-	
Average	19.7	19.4	47.2	45.5		
Source of Variation			***p-value***			
N	<0.0001		<0.0001		-	
(*A.b.*)	0.5335		0.2955		-	
N *x* (*A.b.*)	0.0275		0.5179		-	

† **-/+,** plots were not treated or treated with *Azospirillum brasilense* (*A.b.*).
*Denotes a significant main effect of (*A.b.*).
Yorkville N Accumulation LSD $_{\text{N}\,x\,(\text{A.}b.)}$ (0.1) = 4.71; Champaign N Accumulation LSD $_{\text{N}\,x\,(\text{A.}b.)}$ (0.1) = 5.81; Nashville Biomass LSD $_{\text{N}\,x\,(\text{A.}b.)}$ (0.1) = 1.95.

Figure 2.1 Visual difference in plant growth of plants receiving 100 lbs N/acre and treated with NODa in-furrow (left) and the same rate of N without NODa (right). Picture of V8 plants are at Yorkville, Illinois, in 2020.

Table 2.4 Effect of N and *Azospirillum brasilense* addition on total N uptake, total biomass, and dry weight harvest index at the R6 growth stage of corn at Yorkville, IL in 2020.

Preplant N Rate	**Total N Uptake**		**Total Biomass**		**Harvest Index**	
	Added *Azospirillum brasilense*†					
	-	+	-	+	-	+
	lbs N/acre		grams/plant		%	
0	130	123	206	214	47	49
50	143	143	231	228	50	52
100	169	160	249	242	50	48
150	188	175	280	250	47	48
200	170	182	280	268	47	48
Average	160	157	249	240	48	49
Source of Variation	***p-value***					
N	<0.0001		<0.0001		0.1117	
(*A.b.*)	0.3836		0.2025		0.3853	
N *x* (*A.b.*)	0.2584		0.5334		0.7595	

† **-/+,** plots were not treated or treated with *Azospirillum brasilense* (*A.b.*).
*Denotes a significant main effect of (*A.b.*).

Table 2.5 Effect of N and *Azospirillum brasilense* addition on total N uptake, total biomass, and dry weight harvest index at the R6 growth stage of corn at Champaign, IL in 2019 and 2020.

Preplant N Rate	Total N Uptake		Total Biomass		Harvest Index	
	Added *Azospirillum brasilense*†					
	-	+	-	+	-	+
	lbs N/acre		grams/plant		%	
			2019			
0	62	58	112	112	36	37
50	74	103	150	170	41	39
100	101	94	194	183	46	46
150	111	107	214	198	49	48
200	116	147	224	246	52	51
Average	93	102	178	182	45	44
Source of Variation			*p-value*			
N	<0.0001		<0.0001		<0.0001	
(*A.b.*)	0.1677		0.6723		0.4997	
N *x* (*A.b.*)	0.1691		0.3547		0.9050	
			2020			
0	83	82	168	155	45	47
50	110	120*	197	211	51	50
100	128	137*	215	228	52	53
150	153	156	254	243	51	53
200	156	162*	222	232	52	50
Average	126	131*	211	214	50	51
Source of Variation			*p-value*			
N	0.0001		<0.0001		0.0012	
(*A.b.*)	0.0818		0.5754		0.4950	
N *x* (*A.b.*)	0.7065		0.1776		0.2849	

† **-/+,** plots were not treated or treated with *Azospirillum brasilense* (*A.b.*).
*Denotes a significant main effect of (*A.b.*).

Table 2.6 Effect of N and *Azospirillum brasilense* addition on total N uptake, total biomass, and dry weight harvest index at the R6 growth stage of corn at Nashville, IL in 2020.

Preplant N Rate	**Total N Uptake**		**Total Biomass**		**Harvest Index**	
	Added *Azospirillum brasilense*†					
	-	+	**-**	+	**-**	+
lbs N/acre			grams/plant		%	
0	81	79	164	157	43	44
50	108	114	196	189	49	48
100	135	141	207	206	49	48
150	166	163	241	233	49	51
200	183	169	240	232	51	52
Average	135	133	210	203	48	49
Source of Variation	***p-value***					
N	<0.0001		<0.0001		<0.0001	
(*A.b.*)	0.6616		0.2147		0.6332	
N *x* (*A.b.*)	0.2943		0.9919		0.2192	

† **-/+,** plots were not treated or treated with *Azospirillum brasilense* (*A.b.*).
*Denotes a significant main effect of (*A.b.*).

Table 2.7 Effect of N and *Azospirillum brasilense* addition on corn grain yield, yield components (seed number and seed weight), and grain quality (oil, protein, and starch concentrations) at the R6 growth stage of corn at Yorkville, IL in 2019 and 2020. Grain yield is expressed at 15.5% moisture, while seed weight and grain quality are expressed at 0% moisture.

Preplant N Rate	Grain Yield		Yield Components: Kernel Number		Yield Components: Kernel Weight		Grain Quality: Oil		Grain Quality: Protein		Grain Quality: Starch	
	Added *Azospirillum brasilense*†											
	-	+	-	+	-	+	-	+	-	+	-	+
lbs N/acre	bu/acre		kernels/m^2		mg/kernel		%					
2019												
0	150	152	4155	4112	196	196	4.34	4.35	6.03	5.92	72.9	72.7
50	204	204	5155	4856	210	216	4.13	3.92	6.17	5.98	72.5	73.1
100	214	218	5168	5455	219	213	3.83	3.80	6.42	6.19	73.0	73.3
150	232	232	5538	5557	223	221	3.92	3.94	6.60	6.75	72.9	72.9
200	235	230	5548	5372	224	228	3.56	3.94	6.66	6.88	73.4	72.6
Average	207	207	5113	5070	214	215	3.95	3.99	6.38	6.34	72.9	72.9
Source of Variation	*p-value*											
N	<0.0001		<0.0001		<0.0001		<0.0001		<0.0001		0.6308	
(*A.b.*)	0.9220		0.3256		0.9603		0.6458		0.5701		0.9190	
N *x* (*A.b.*)	0.9596		0.0846		0.4820		0.1698		0.4488		0.1561	
2020												
0	162	159	3551	3517	241	241	4.33	4.51	7.52	7.75*	72.7	72.4
50	182	184	3747	3859	258	253	4.39	4.28	7.83	7.77	72.2	72.5
100	193	184	3892	3857	263	253	4.36	4.41	8.10	8.32*	72.4	72.1
150	193	190	3878	3758	264	268	4.53	4.47	8.40	8.45	71.7	71.7
200	188	192	3661	3839	274	264	4.56	4.76	8.50	8.65*	71.6	71.6
Average	184	182	3742	3766	260	256	4.44	4.49	8.07	8.19*	72.1	72.1
Source of Variation	*p-value*											
N	0.0151		0.2364		0.0010		0.0067		<0.0001		<0.0001	
(*A.b.*)	0.5998		0.7174		0.1333		0.3726		0.0297		0.5205	
N *x* (*A.b.*)	0.7256		0.6013		0.5139		0.2892		0.3022		0.4560	

† **-/+,** plots were not treated or treated with *Azospirillum brasilense* (*A.b.*).

*Denotes a significant main effect of (*A.b.*).

Table 2.8 Effect of N and *Azospirillum brasilense* addition on corn grain yield, yield components (seed number and seed weight), and grain quality (oil, protein, and starch concentrations) at the R6 growth stage of corn at Champaign, IL in 2019 and 2020. Grain yield is expressed at 15.5% moisture, while seed weight and grain quality are expressed at 0% moisture.

Preplant N Rate	Grain Yield		Yield Components: Kernel Number		Yield Components: Kernel Weight		Grain Quality: Oil		Grain Quality: Protein		Grain Quality: Starch	
	Added *Azospirillum brasilense*†											
	-	+	-	+	-	+	-	+	-	+	-	+
lbs N/acre	bu/acre		kernels/m^2		mg/kernel		%					
2019												
0	65	67	1962	2093	170	169	4.09	4.09	5.73	5.77	73.8	73.8
50	98	107	2853	3620	181	182	4.27	4.28	5.80	5.97	73.9	73.8
100	160	138	3972	3631	195	193	3.97	4.18	5.79	5.92	73.8	74.1*
150	170	153	4200	4118	212	205	4.06	4.07	5.90	5.83	73.4	74.4*
200	197	204	4716	4614	227	232	4.09	4.03	6.37	6.17	73.3	73.5
Average	142	137	3541	3615	197	196	4.10	4.13	5.92	5.93	73.6	73.9
Source of Variation	*p-value*											
N	<0.0001		<0.0001		<0.0001		0.0489		0.0007		0.1364	
(*A.b.*)	0.5085		0.4486		0.7061		0.5405		0.8896		0.0671	
N *x* (*A.b.*)	0.4040		0.0165		0.2720		0.5274		0.4790		0.1737	
2020												
0	115	116	3007	3107	202	198	4.08	4.01	6.08	5.80	73.7	74.4
50	158	164	3838	3993	218	218	4.06	3.97	6.12	5.98	74.0	74.2
100	179	181	3759	3676	230	234	4.16	4.01	6.60	6.63	73.3	73.7
150	189	196	4089	4181	232	238	3.94	4.10	6.68	6.73	73.6	73.3
200	178	175	4187	4063	225	226	4.09	4.12	6.83	6.98	73.4	73.4
Average	164	166	3776	3804	221	223	4.07	4.04	6.46	6.43	73.6	73.8
Source of Variation	*p-value*											
N	<0.0001		0.0260		<0.0001		0.6914		<0.0001		0.0006	
(*A.b.*)	0.2706		0.6075		0.5436		0.6100		0.5502		0.1340	
N *x* (*A.b.*)	0.5596		0.3932		0.7926		0.2116		0.2052		0.1225	

† **-/+,** plots were not treated or treated with *Azospirillum brasilense* (*A.b.*).

*Denotes a significant main effect of (*A.b.*).

2019 Kernel Number LSD $_{\text{N } x\ (A.b.)}$ (0.1) = 369.

Table 2.9 Effect of N and *Azospirillum brasilense* addition on corn grain yield, yield components (seed number and seed weight), and grain quality (oil, protein, and starch concentrations) at the R6 growth stage of corn at Ewing, IL in 2019 and at Nashville, IL in 2020. Grain yield is expressed at 15.5% moisture, while seed weight and grain quality are expressed at 0% moisture.

Preplant N Rate	Grain Yield		Yield Components: Kernel Number		Yield Components: Kernel Weight		Grain Quality: Oil		Grain Quality: Protein		Grain Quality: Starch	
	Added *Azospirillum brasilense*†											
	-	+	-	+	-	+	-	+	-	+	-	+
lbs N/acre	bu/acre		kernels/m^2		mg/kernel		%					
2019												
0	94	106	3009	3446	164	163	3.92	3.86	6.09	5.64*	73.2	73.8
50	115	123	3622	3686	169	176	3.79	3.84	6.14	5.97*	73.7	73.4
100	153	137	4196	4288	195	169	3.71	3.72	6.03	5.66*	73.3	73.7
150	146	133	4187	3795	185	185	3.75	3.71	5.86	5.83	73.5	73.8
200	138	139	4071	4363	179	186	3.84	3.82	6.48	6.46	73.0	73.3
Average	129	128	3817	3916	179	176	3.80	3.79	6.12	5.91*	73.4	73.6
Source of Variation	*p-value*											
N	0.0085		0.0039		0.0623		0.3092		0.0056		0.3864	
(*A.b.*)	0.8269		0.5526		0.5739		0.8313		0.0546		0.1332	
N *x* (*A.b.*)	0.7460		0.5385		0.2710		0.9750		0.6187		0.4644	
2020												
0	118	109	3195	3052	196	190	3.88	3.98	6.23	6.33	74.4	74.3
50	152	153	3814	3784	211	215	4.13	4.03	6.75	6.68	73.8	74.0
100	175	174	4219	4091	220	226	3.86	3.83	6.63	6.55	74.1	74.2
150	178	179	4150	4107	228	232	3.82	3.90	6.75	6.95	73.8	73.8
200	191	193	4217	4338	241	237	3.88	3.99	7.27	7.23	73.6	73.6
Average	163	162	3919	3874	219	220	3.91	3.95	6.73	6.75	73.9	74.0
Source of Variation	*p-value*											
N	<0.0001		<0.0001		<0.0001		0.3492		0.0004		0.0162	
(*A.b.*)	0.6278		0.4419		0.7766		0.5420		0.7529		0.9078	
N *x* (*A.b.*)	0.3427		0.6102		0.3624		0.7119		0.7033		0.9911	

† **-/+,** plots were not treated or treated with *Azospirillum brasilense* (*A.b.*).

*Denotes a significant main effect of (*A.b.*).

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APPENDIX A: SUPPLEMENTAL TABLES

Table A.1. Effect of foliar treatments and plant population on grain quality (oil and protein concentrations) for soybean grown at Yorkville, IL in 2019. Grain qualities are expressed at 13% moisture.

Foliar Treatments		**Plant Population,** plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		Oil, %			
None	-	17.9	17.7	17.6	17.7
None	Added	18.0	17.8	17.8	17.9*
V5	-	17.9	17.8	17.8	17.8
R3	-	17.9	17.9	17.7	17.8
R3	Added	18.0	17.9	17.9	17.9*
V5 + R3	-	18.0	18.0	17.7	17.9*
V5 + R3	Added	18.0	17.9	17.8	17.9*
Average		18.0	17.9	17.8	
		Protein, %			
None	-	34.6	34.7	34.8	34.7
None	Added	34.1	34.5	34.5	34.4*
V5	-	34.4	34.5	34.8	34.6
R3	-	34.4	34.6	34.8	34.6
R3	Added	34.1	34.6	34.5	34.4*
V5 + R3	-	34.4	34.6	34.8	34.6
V5 + R3	Added	34.2	34.3	34.8	34.5*
Average		34.3	34.5	34.7*	

Source of Variation	Oil	Protein
	p-value	
Foliar Treatments	0.0637	0.0179
Plant Population	0.3835	0.0988
Plant Pop. *x* Foliar Treat.	0.7382	0.6148

*Denotes a significant main effect compared to no treatment or 80,000 plants acre^{-1}.

Table A.2. Effect of foliar treatments and plant population on grain quality (oil and protein concentrations) for soybean grown at Champaign, IL in 2019. Grain qualities are expressed at 13% moisture.

Foliar Treatments		**Plant Population,** plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		Oil, %			
None	-	19.4	19.2	19.1	19.2
None	Added	19.3	19.1	19.0	19.2
V5	-	19.4	19.1	19.0	19.1*
R3	-	19.4	19.2	19.0	19.2
R3	Added	19.5	19.2	19.0	19.2
V5 + R3	-	19.6	19.2	19.1	19.3
V5 + R3	Added	19.6	19.2	19.1	19.3
Average		19.4	19.2*	19.0*	
		Protein, %			
None	-	33.5	34.0	34.3	33.9
None	Added	33.5	34.0	34.3	33.9
V5	-	33.5	34.0	34.3	33.9
R3	-	33.3	33.7	34.2	33.7*
R3	Added	33.4	33.8	34.0	33.7*
V5 + R3	-	33.2	33.9	34.1	33.7*
V5 + R3	Added	33.3	34.0	34.1	33.8
Average		33.4	33.9*	34.2*	

Source of Variation	Oil	Protein
	p-value	
Foliar Treatments	0.0311	0.0066
Plant Population	0.0030	0.0008
Plant Pop. *x* Foliar Treat.	0.9315	0.6071

*Denotes a significant main effect compared to no treatment or 80,000 plants acre^{-1}.

Table A.3. Effect of foliar treatments and plant population on grain quality (oil and protein concentrations) for soybean grown at Ewing, IL in 2019. Grain qualities are expressed at 13% moisture.

Foliar Treatments		**Plant Population,** plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		Oil, %			
None	-	20.0	20.0	19.7	19.9
None	Added	20.3	20.1	19.9	20.1*
V5	-	20.2	19.9	19.8	19.9
R3	-	20.1	20.0	19.7	19.9
R3	Added	20.1	20.0	19.9	20.0*
V5 + R3	-	20.4	20.0	19.7	19.9
V5 + R3	Added	20.4	20.0	19.9	20.1*
Average		20.2	20.0*	19.8*	
		Protein, %			
None	-	33.9	34.5	35.0	34.5
None	Added	33.9	34.4	34.8	34.4
V5	-	34.0	34.6	34.9	34.5
R3	-	34.2	34.6	34.9	34.6
R3	Added	33.9	34.3	34.7	34.3*
V5 + R3	-	34.1	34.4	35.0	34.5
V5 + R3	Added	33.7	34.4	34.6	34.2*
Average		33.9	34.5*	34.9*	

Source of Variation	Oil	Protein
	p-value	
Foliar Treatments	<0.0001	0.0005
Plant Population	0.0010	<0.0001
Plant Pop. *x* Foliar Treat.	0.3339	0.4705

*Denotes a significant main effect compared to no treatment or 80,000 plants acre^{-1}.

Table A.4. Effect of foliar treatments and plant population on grain quality (oil and protein concentrations) for soybean grown at Yorkville, IL in 2020. Grain qualities are expressed at 13% moisture.

Foliar Treatments		**Plant Population,** plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		Oil, %			
-	-	18.2	18.2	18.2	18.2
V5	-	18.2	18.1	18.2	18.2
V5	Added	18.1	18.2	18.1	18.1
R3	-	18.2	18.1	18.1	18.1
R3	Added	18.2	18.2	18.1	18.2
V5 + R3	-	18.2	18.0	18.1	18.1
V5 + R3	Added	18.2	18.1	18.1	18.1
Average		18.2	18.1	18.1	
		Protein, %			
-	-	34.0	34.1	34.3	34.1
V5	-	33.9	34.2	34.2	34.1
V5	Added	33.8	33.9	34.1	33.9*
R3	-	33.8	34.2	34.3	34.1
R3	Added	33.9	34.1	34.2	34.1
V5 + R3	-	34.0	34.3	34.3	34.2
V5 + R3	Added	33.8	34.1	34.1	34.0*
Average		33.9	34.1*	34.2*	

Source of Variation	Oil	Protein
	p-value	
Foliar Treatments	0.7160	0.0034
Plant Population	0.5261	0.0406
Plant Pop. *x* Foliar Treat.	0.7086	0.8531

*Denotes a significant main effect compared to no treatment or 80,000 plants acre^{-1}.

Table A.5. Effect of foliar treatments and plant population on grain quality (oil and protein concentrations) for soybean grown at Champaign, IL in 2020. Grain qualities are expressed at 13% moisture.

Foliar Treatments		**Plant Population,** plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		Oil, %			
-	-	19.9	19.8	19.7	19.8
V5	-	20.0	19.7	19.7	19.8
V5	Added	20.1	19.9	19.7	19.9
R3	-	20.0	19.8	19.8	19.9
R3	Added	19.8	19.8	19.7	19.8
V5 + R3	-	20.0	19.9	19.7	19.9
V5 + R3	Added	20.0	19.8	19.7	19.8
Average		20.0	19.8*	19.7*	
		Protein, %			
-	-	34.2	34.2	34.2	34.2
V5	-	34.2	34.2	34.3	34.2
V5	Added	34.0	33.9	34.2	34.0
R3	-	34.0	34.1	34.2	34.1
R3	Added	34.1	34.0	33.9	34.0
V5 + R3	-	34.0	34.0	34.3	34.1
V5 + R3	Added	34.0	34.3	34.2	34.2
Average		34.1	34.1	34.2	

Source of Variation	Oil	Protein
	p-value	
Foliar Treatments	0.0387	0.1747
Plant Population	0.0005	0.5247
Plant Pop. *x* Foliar Treat.	0.3220	0.2322

*Denotes a significant main effect compared to 80,000 plants acre^{-1}.

Table A.6. Effect of foliar treatments and plant population on grain quality (oil and protein concentrations) for soybean grown at Nashville, IL in 2020. Grain qualities are expressed at 13% moisture.

Foliar Treatments		Plant Population, plants acre^{-1}			
AIPGR Application	**Foliar Protection**	**80,000**	**140,000**	**200,000**	**Average**
		Oil, %			
-	-	19.8	20.0	19.7	19.8
V5	-	19.8	19.8	19.6	19.7*
V5	Added	20.0	20.0	19.9	20.0*
R3	-	19.9	19.9	19.7	19.8
R3	Added	20.1	19.9	19.8	20.0*
V5 + R3	-	19.9	19.9	19.9	19.9*
V5 + R3	Added	20.0	20.0	19.8	19.9*
Average		19.9	19.9	19.8	
		Protein, %			
-	-	35.0	34.9	35.3	35.1
V5	-	35.1	35.2	35.4	35.2*
V5	Added	34.9	34.8	35.0	34.9*
R3	-	34.9	35.0	35.3	35.1
R3	Added	34.7	35.0	35.1	35.0
V5 + R3	-	34.8	34.9	35.2	35.0
V5 + R3	Added	34.8	34.9	35.2	35.0
Average		34.9	35.0	35.2	

Source of Variation	Oil	Protein
	p-value	
Foliar Treatments	<0.0001	0.0011
Plant Population	0.4464	0.2469
Plant Pop. *x* Foliar Treat.	0.2183	0.2007

*Denotes a significant main effect compared to no treatment or 80,000 plants acre^{-1}.